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A Cytological Study of the Progeny of X-rayed *Crepis capillaris* Wallr.

By

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In the summer of 1929 there appeared the remarkable papers of PAINTER and MULLER, on the one hand, and of DOBZHANSKY, on the other, about the discovery of the translocation of segments from one chromosome to another as the result of X-radiation. Upon reading these papers, I at once, that very fall, commenced experiments on the X-radiation of *Crepis capillaris*, a classical plant as regards its chromosome set, which consists of three pairs of homologues (A, C, D) clearly distinguishable from one another (Fig. 1). The diverse chromosome changes occurring in seedlings of *Crepis*, vetch, and rye subjected to X-radiation were treated in a special paper (LEWITSKY and ARARATYAN, 1931). Somewhat earlier a similar paper on *Crepis tectorum* had been published by M. NAVASHIN (1931).

In the spring of 1930 another lot of *Crepis capillaris* seedlings were subjected to X-radiation. Out of the mature plants 20 individuals were selected which proved sharply deviating from the remaining majority of rather normal ones. The seeds collected from these free pollinated plants gave rise to the F_1 progeny of X-rayed plants.[1)]

The cytological study of this progeny was begun in the fall of 1930. From each plant several root-tips were fixed and investigated. However, later (with the F_2) we changed to a new method of "mass cytological investigation" which I had developed (LEWITSKY, 1932), consisting in cutting off the primary root-tips and pasting them in groups on pieces of paper, so as later to fix them all at once and complete the preparation of the slides.

The seedlings whose root-tips had been removed were transplanted to small pots labelled with the same numbers as the corresponding root-tips and placed in a cool greenhouse under electric illumination, where, for the most part, they quite rapidly formed a new root system and developed normally. Meanwhile the cytological investigation of the root-tips was in progress, and by the end of

1) In addition we had F_1 plants grown from seed taken from three X-rayed plants whose buds were of different ages at the time of irradiation.

spring it was possible to give a karyological characterization of many of the plants. A preliminary communication on new karyotypical races occurring in the progeny of X-rayed plants was published by

Table 1. Progeny (F_1) of X-rayed

1	2	3	4	5	6	7	8	9	10	11	12	13
Serial No.	Dosage	Pedigree no. of X-rayed plant	Seedlings set out	Seedlings died	Cytologically investigated	Normal	Aberrant	Translocation "from D to C"	Translocation "from D to A"	Translocation "from A to C"	"Constriction shifting" in A (A_{sh})	"Constriction shifting" in C (C_{sh})
1	I-30	2503	35	19	16	15	1			1		
2	I-60	2461	10	2	8	3	5		3			
3	,, ,,	2465	28	7	21	20	1			1		
4	,, ,,	2470	40	11	28	24	4			1		
5	,, ,,	2473	36	10	22	19	3			1	1	
6	II-5	2475*	74	—	32	30	2	1				
7	II-20	2500*	16	—	16	16	—					
8	II-30	2501*	50	25	23	21	2				2	
9	II-40	2468	27	6	21	18	3				1	
10	,, ,,	2472	28	6	18	14	4	2		1		1
11	,, ,,	2533	56	6	48	39	9			6		
12	,, ,,	2534	24	4	13	4	9			8		
13	,, ,,	2535	15	—	11	8	3			1		
14	,, ,,	2536	29	1	25	20	5					
15	III-3	2469	33	12	21	17	4					
16	III-6	2464	36	12	13	13	—					
17	,, ,,	2466	28	11	14	13	1					
18	,, ,,	2471	33	10	22	18	4			1		
19	,, ,,	2491*	28	17	9	9	—					
20	,, ,,	2532	83	5	72	66	6					
21	III-10	2467	35	13	17	4	13			1		
22	,, ,,	2474	36	13	19	14	5		2	1		
23	,, ,,	2474a	29	13	2	2	—					
		Total	809	203	491	407	84					
Number of aberrant plants of each type								3	5	23	4	1
No. of parent plants giving origin to each aberrant type								2	2	11	3	1

* X-rayed during stage of budding; all others X-rayed during seedling stage.

us in 1934 (LEWITSKY, SHEPELEVA and TITOBA, 1934).

All the data on the origin and analysis of our first generation (F_1) are given in Table 1. In this table are listed the initial

Crepis capillaris WALLR.

14	15	16	17	18	19	20	21	22	23	24	25	26	27
Unequal heads in C	Shortened short arm of A and C	D inversion (D_{inv})	D+x	Translocation of short arm of A to satell. D	+(D−d)	Translocation "from D to A" and C_{sh}	Translocation "from A to C" and D+x	A_{sh} and C_{sh}	3n	4n	3n+translocation	Trisomic	Trisomic and translocation "from A to C"
					1	1							
1									1	1			
					1								
1													
	1							1					
		1							1		1D→A		
											1D→A		
1			1										
2				2			1						
2									2				
1													
3													
1					1				2		1D→C	1(+A)	
1		5	2				1				1D→D	2(+C)(+D)	
1													1(+C)
14	1	6	3	2	3	1	2	1	6	1	4	3	1
10	1	2	2	1	3	1	2	1	4	1	4	2	1

1) For details see our earlier paper (LEWITSKY and ARARATYAN, 1931).

"parental" plants, subjected to X-radiation during the seedling stage, and the respective dosages[1] and pedigree numbers are indicated. The table then gives the number of seedling progeny set out from each initial plant, the number which died and the number cytologically investigated, indicating the number of normal and aberrant individuals, and, lastly, a characterization of the separate aberrations with the number of each.

Out of 491 investigated F_1 plants 84 showed aberrations, i.e., 17.1 per cent.[1] Most of them (74) were so-called "structural" chromosome changes—either unaccompanied by other alterations (69) or in combination with triploidy (4) or a trisomic condition (1). There were ten cases of simple numerical changes, *i.e.*, 6 triploids, 1 tetraploid, and 3 trisomics of all the three possible types.

The most common type of cytologically established structural chromosome changes are translocations, characterized by a shortening of one choromosome and a corresponding lengthening of another. In our preliminary communication of 1934 such changes were treated as a simple transfer of a segment of one chromosome to another chromosome. The same interpretation was made in setting forth the regularities observed in chromosome changes induced by X-rays (LEWITSKY and SIZOVA, 1934). We were impelled to such a point of view by the investigation made in our laboratory (PETROV, 1935) of meiosis in the karyotypical aberrants of *Crepis capillaris* I had obtained, in which investigation there were found at diakinesis of heterozygous "translocants" exclusively the chain-like configurations of chromosomes characteristic of simple translocations.

In connection with this problem we undertook a more detailed analysis of our material (by then considerably more extensive) on structural chromosome changes which had been treated in our earlier paper (LEWITSKY and SIZOVA, 1934). From this second analysis we obtained indubitable proof that "most (and perhaps all) X-ray translocations in *Crepis* are reciprocal" (LEWITSKY and SIZOVA, 1935). Furthermore, a second investigation (KORYUKAYEV, in press), conducted at earlier meiotic stages, of the aberrant material analyzed by PETROV showed the reciprocal nature of two of our chief translocations, though ring configurations occurred rarely, due, presumably, to the extremely small size of one of the interchanged segments (cnf also GERASSIMOVA, 1939). Cases of reciprocal translocation were also reported by M. NAVASHIN and E. GERASSIMOVA for *Crepis tectorum* (1936). In all cases, of course, there are revealed in somatic plates

1) In our preliminary communication (1934), in which only part of the material had been analyzed, data were given for only 272 F_1 plants; of these 46, i.e., also about 17 per cent, were aberrants.

only those translocations in which the "interchange" has occurred between noticeably unequal segments, causing thereby a more or less marked shortening of one chromosome and a corresponding lengthening of the other. Henceforth, we shall have this reciprocity in mind, although we shall continue to speak—for sake of convenience—about translocations "from one chromosome to another." Other types of deviations we shall consider in consecutive order.

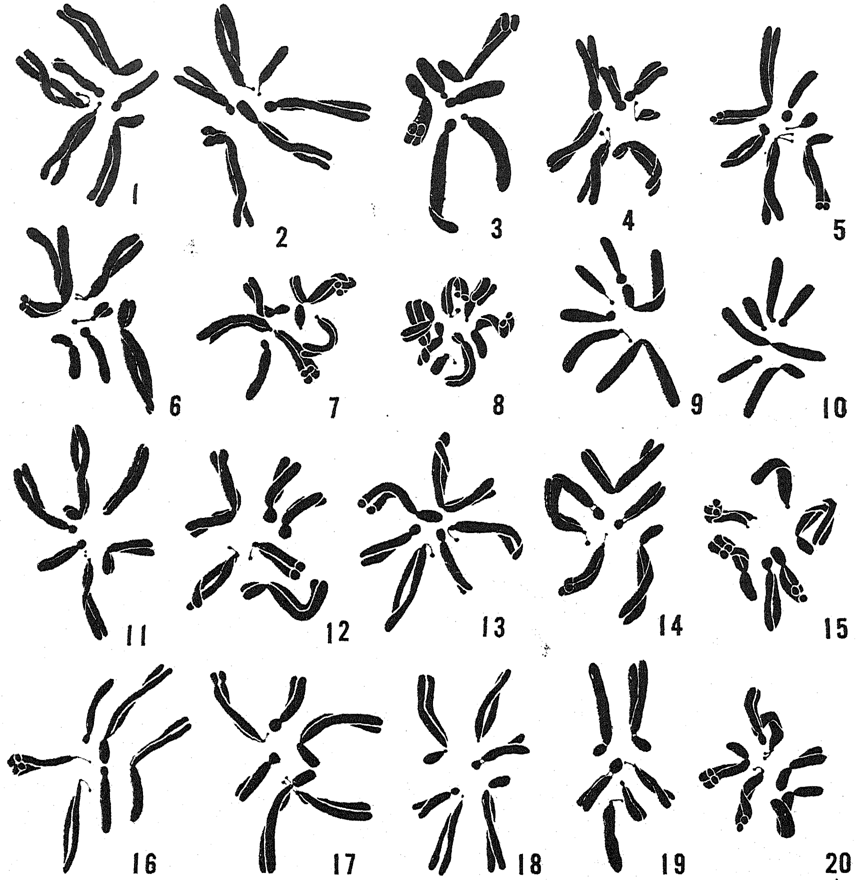
From each of the X-rayed parental plants there were in most cases obtained several aberrant individuals. The deviations, in general, varied, but in many cases a considerable number and sometimes even the majority of plants arising from the same parent were characterized by a single type of deviation. All the structural chromosome changes were in a heterozygous state, i.e., the change affected only one chromosome, or, if two, then non-homologous ones.

Of translocations we obtained: "from D to C", i.e., from the long arm of D to the long arm of C; "from D to A", i.e., from the long arm of D to the short arm of A; and "from A to C", i.e., from the long arm of A to the long arm of C. The translocation "from D to C" was obtained from two parental plants, one of which (2475) was X-rayed during the stage of budding and the other (2472) during the seedling stage. The changes in the two cases were similar (Figs. 2 and 3). The translocation "from D to A" was also obtained from two plants, both X-rayed as seedlings: from 2461 (No. 2) three individuals and from 2474 (No. 22) two. The first three are shown in Figures 4, 5 and 6 and seem to be identical. From the same initial plant there were obtained: (1) one plant (Fig. 7) with the same translocation (the change in D being *identical to the first two simple translocations*—Figs. 4 and 5) combined with a "shift of the attachment constriction" in C (C_{sh}) and (2) one plant (Fig. 8) with an additional chromosome D-d being of the SAME SIZE as the analogous little chromosome in Figures 4, 5, 6 and 7.

Both translocants arising from the other X-rayed plant (2474) were characterized by a translocation between the same chromosomes but *clearly of another kind,* the shortened D being considerably longer (Figs. 9 and 10) than in the previous case. The two individuals, however, very closely resembled each other, as may be seen from the figures.

A simple translocation "from A to C" (Fig. 11) occurred in 23 individuals originating from 11 X-rayed plants. In three other individuals the same translocation was accompanied by other changes (Table 1, Cols. 21, 27). Seven of the X-rayed plants each gave one plant with this translocation, two gave two each (Nos. 21 and 22), and the other two—6 and 8, respectively.

Measuring of the altered A-chromosomes in seven single different aberrant individuals gives following ratios between the two arms: 2503—2.14, 2465—2.12, 2472—2.06, 2536—1.95, 2470—1.71, 2474—1.62, 2535—1.49.



Figs. 1–20. **1.** Normal nuclear plate of *Crepis capillaris* WALLR. **2, 3.** Translocation "from D to C" (two individuals from the mother plants 2475 and 2472). **4–7.** Translocation "from D to A" (four individuals from one mother-plant 2461). In the Fig. 7- a "constriction shifting" in the chromosome C. **8.** An additional proximal fragment of the chromosome D (the same mother plant 2461). **9, 10.** Translocation "from D to A" (two individuals from one mother plant 2474). **11.** Translocation "from A to C". **12, 13.** "Constriction shifting" in A-chr. (two individuals from one mother plant 2501). **14.** "Constriction shifting" in A-chr. (mother-plant 2468). **15.** Elongation of the short arm in A- and C-chrom. (mother-plant 2467). **16.** "Constriction shifting" in A-chr. (mother-plant 2473). **17, 18.** Unequal heads in C-chr. **19.** Diminution of the short arm in A- and C chr. **20.** "Constriction shifting" in C-chr.

Let us now examine the groups of aberrants arising from single irradiated mother plants. From Plant 2467 (No. 21) one of the aberrants is a simple translocation. The other aberrant combines

the same translocation with a large extra appendage on D(D+x, col. 21). The relative lengths of the arms in both aberrants is almost the same (1.9).

Two of the X-rayed plants (2533 and 2534—Nos. 11 and 12) gave a *considerable* number of similar translocations "from A to C", viz., 6 and 8, respectively. The first of these plants also gave rise to the following aberrants: alteration in the D-chromosome, triploid, and triploid with a translocation "from D to A"; and the second—only to one triploid with a translocation "from D to A". Let us first examine the translocations in the progeny of Plant 2533. The shortened A-chromosomes in its four[1] aberrants, despite slight differences, due to a contraction, are very similar as regards the ratio between the two arms (2.0, 2.0, 2.14, 2.2). Let us pass to the progeny of Plant 2534. The shortened A-chromosomes of two[1] of its aberrants are also very similar as regards the ratio between the two arms (2.44, 2.5).

Thus, we see that translocations of the same type in individuals originating from *different* initial plants may be *very different,* while translocations of the same type *in the progeny of a single X-rayed plant* are, in general, *very similar.* The same phenomenon, as we have seen, occurred in case of the translocations "from D to A", which were encountered only in several individuals of the same progeny (from Plants Nos. 2 and 22, Figs. 4–10). Such a characteristic of the occurrence of aberrations is particularly patent in case of rare and unique changes, such as translocations of a part of an arm of one D-chromosome to its own satellite (D_{inv}—Table 1, col. 16) which occurred in two progenies, in one of them (2467) five times (see below, p. 10).

All the phenemena just described are quite naturally explained by the fact that chromosomal aberrations occurring in embryonic tissue of a seedling are subsequently spread by cell division to whole sectors of the plant (M. NAVASHIN, 1931; LEWITSKY and ARARATYAN, 1931; KAKHIDZE, 1932), embracing finally whole flowers and inflorescences. The identical aberrant gametes formed by the latter in large number produce zygotes with the same kind of aberration. Thus, translocations characterizing various individuals of one and the same progeny are not merely similar but in reality identical, due to their having the same origin.

Variation in the length of the shortened (due to translocation) long arm of A in our material was to a certain extent limited, the ratio between the arms ranging from 1.49 to 2.5, whereas its possible

1) The slides for the rest were not conserved.

limits are 0 and 3.45 (6:1.6). This is in agreement with the data obtained in investigating a great number of translocations "from A to the long arms of A, C and D" (LEWITSKY and SIZOVA, 1935). These data gave the limits of variation of this ratio as 1.0 and 2.6.

The other deviations are characterized by a change only in one chromosome of the set, which undergoes a definite redistribution of its parts. We shall take up first the so-called "shift of the attachment constriction" in chromosome A (Figs. 12, 13, 14, 15, 16). This aberration is characterized by the fact that, while the total length of the chromosome remains the same, the relative length of its arms changes, the short arm becoming longer and the long arm correspondingly shorter. In Figures 12 and 13 this deviation is shown in its most typical form, when the A-chromosome becomes V-shaped, only slightly unequal-armed. Measurement of the two A-chromosomes in Figure 12 gave the following ratios:[1)]

	Ratio of arms
Normal A-chrom. 5.75+2.0=7.75	2.87 : 1
Altered A-chrom. 4.1+2.5=7.6	1.17 : 1

The plates shown were taken from two individuals in the progeny of Plant 2501 (No. 8), which was X-rayed at the stage of budding. Judging by their similarity, they arose from an aberrant sector of the archesporium, which produced identically altered gametes.

In the next figure (14) chromosome A is already more clearly unequal-armed. The ratio between the arms is 1.29:1 (4.5:3.5). The initial plant was 2468 (No. 9). The same plant produced an individual with a very slight lengthening of the short arm of A, accompanied by a marked lengthening of the short arm of C (Fig. 15). The difference between these two plants was due either to two different aberrations in the mother plant or to fertilization by an aberrant pollen grain.

The fifth individual with a "shift of the attachment constriction" in A, originating from Plant 2473 (No. 5), was characterized by a change in A intermediate between the last two aberrations (ratio between the arms—1.67:1) (Fig. 16).

The abberation just described was investigated as regards the conjugation of the two components of the pair, the normal A-chromosome and the altered A-chromosome, the latter in its most typical, almost equal-armed form. According to the investigations of PETROV (1935), these chromosomes form at diakinesis a normal

1) All the measurements cited in this paper were made by E. M. SHEPELEVA.

bivalent with chiasmata, conjugating sometimes end to end, sometimes in their median sectiens. PETROV concluded, therefore, that the order of the genes, despite the change in the chromosomes, had remained unaltered, the change consisting apparently only in a "shift of the constriction." A reinvestigation of this problem by KORYUKAYEV (unpubl.), with the inclusion of a study of earlier stages, led this investigator to the conclusion that we have to do here with an "internal inversion" of unequal segments of the short and long arms.

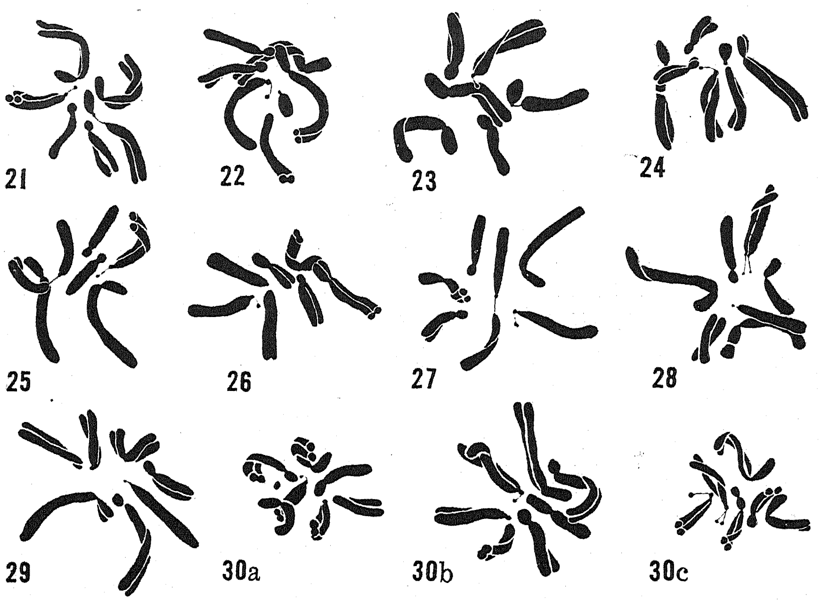
Very often in the present investigation there were observed individuals with a change in the short arm, or head, of C; sometimes it was longer than normal, sometimes shorter (Figs. 17 and 18). The nature of such changes—which, for the sake of being more objective, we designated as "unequal heads in C" (col. 14), is in general not apparent. To observe even a small loss or addition to the "head" was not difficult, but to establish a corresponding lengthening or shortening of the long arm of the same chromosome was, for the most part, impossible, as also to reveal analogous changes in other chromosomes, in case of the transfer to or withdrawal from them of such small segments. In one plant the head of one C-chromosome was shortened to an exceptional degree (Fig. 19). At the same time there occurred a shortening of the short arm of one of the A-chromosomes.

A more considerable "shift of the attachment constriction" in C (col. 13) was observed by us without any doubt only in two plants. Regarding one of them we have already mentioned (Fig. 7); the other is shown very clearly in Figure 20. The nature of this alteration has not been investigated. Presumably there has also taken place here, as in the case of the A-chromosome, an internal inversion.

The next type of rearrangement within the limits of a single chromosome is fcund in chromosome D, and consists of a marked shortening of its long arm, accompanied by the appearance on this much reduced chromosome of a gigantic "satellite," corresponding in size to the segment lost from the long arm (Fig. 21). Such an alteration has been interpreted by us either as an inversion of a proximal segment or (which is essentially the same) as a translocation of a large distal segment to the satellite. It is possible that even here the translocation is reciprocal. In such case the satellite (or an end portion of it) must have been transferred to the "point of breakage" of the shortened D-chromosome, while a distal segment of the long arm was transferred to the place of the satellite (or its end portion). The possibility of such a rearrangement has recently been established by M. NAVASHIN and E. GERASSIMOVA (1936).

In some plates no connecting thread is visible, but that some connection exists is indicated, nevertheless, by the close proximity of these two parts of chromosome D (Fig. 22).

The two plates just described are from a plant belonging to the progeny of the X-rayed plant 2467 (No. 21). In all this plant produced five such aberrants. In Figure 23 is shown a plate from



Figs. 21–30. **21–24.** Inversion of proximal portion in D-chr (or a translocation of a distal portion of D-chr. to the satellite of the same chromosome). Mother-plant 2467. **21, 22.** Individual 2467/21. **23.** Individual 2467/15. **24.** The segments of chromos. D approximated. Individual 2467/10. **25.** D-inversion from another mother-plant (2533). **26–30.** A big satellite on the chromosome D. A thread is seen only on the plate of Figures 27. **26, 28.** From one individual with a constriction shifting in C-chr. **29.** From another individual, but from the same mother-plant (2467) (characterized by translocation "from A to C"). **27.** Third individual from the same mother-plant (2467). **30.** Plant from maternal parent No. 2536 (2536/23). Phenomena of elimination of elements from the chromosome set: a—"Appendage" on chromosome D and additional, miniature, independent chromosome without visible articulation; translocation "from A to C"; b—Additional miniature chromosome missing; c—"Appendage" missing; satellites on both D-chromosomes.

another specimen (2467/15). The similarity of the two aberrations is striking. In two other specimens it is represented in precisely the same form. In the fifth aberrant (2467/10) both parts of the altered D-chromosome are so very close together that it assumes a resemblance to A (Fig. 24).

In sharp contrast with these quite similar aberrations among

the progeny of one parental plant, is an aberration of the same type but originating from another parental plant (2533—No. 11). In this case, as the result of the alteration, chromosome D appeared divided into two, almost equal segments, united by a thread attached to the head of one of them (Fig. 25).

The next two types of deviations comprise *additions of extra elements* to the normal set. The first of these (D+x), originating from the X-rayed plant 2467 (No. 21—$\frac{2467}{6}$, Col. 21) resembles to a certain extent the aberration just described, viz., one of the D-chromosomes, instead of its usual small satellite, is accompanied by an enormous "fragment," sometimes as large as the chromosome itself (Fig. 26). A connecting thread may be seen only in rare cases (Fig. 27), but the constant proximity of one end of the "fragment" to one end of the D-chromosome indicates that they are really connected. The "fragment" thus proves to be a gigantic satellite. The essential difference between this and the preceding aberration lies in the fact that in this case the aberrant D-chromosome (including its gigantic satellite) is much larger than its homologue. Other peculiarities of this deviation (Fig. 26) are: (1) "shift of the attachment constriction" in one of the C-chromosomes and (2) shortening of the long arm of both D-chromosomes as compared with A. This last peculiarity is shown in Figure 28, taken from the same specimen (2467/6). Here attention is also drawn to the inconsiderable size (as compared with Fig. 26) of the "fragment"—a case of "intra-individual variation" (LEWITSKY, 1937). In a sister plant (2467/5) the same deviation was observed, but both C-chromosomes were normal (Fig. 27). This same peculiar aberration of chromosome D was also revealed in the same progeny (of Plant 2467), in combination with a translocation "from A to C" (col. 21) in its characteristic form (Fig. 29). The aberrant D-chromosome—just as in the plate shown in Figure 26—is considerably shorter than the long arm of chromosome A and, in this case, even shorter than its own gigantic satellite.

Analogous deviations were found by us in another plant belonging to the progeny of Plant 2535 (No. 17) and, in combination with a translocation "from A to C" (col. 21), in the progeny of Plants 2536 (No. 23) (Fig. 30b).

It is interesting that the presence of such an enormous satellite, attached by a slender thread, leads sometimes to its breaking off from the chromosome and to its complete loss from the set. In one of the specimens with a "fragment" just described I found two plates without any traces of this "fragment," and in another the fragment

was revealed torn off and separated from the plate.[1] The origin of such "augmentative aberrations" is evidently connected with the translocation to D's satellite and with the coming together in the zygote of a normal and an aberrant D-chromosome. Whence might this translocated segment have come? Judging by its size, only from D or A. In the latter case the most probable would be balanced gametes D+a and A+a (cf. ARENKOVA, 1939). The absence of such gametes—in general less viable—in our five cases of gametes with "augmentative aberrations" points to a translocation D−d/D+d, resulting in *exclusively* unbalanced gametes. Gametes with a loss (D−d) are very feeble (ARENKOVA, l.c.), the few surviving gametes with an excess will result in our aberration.

The most interesting phenomena were observed in one plant, plates from which are shown in Figure 30. Two plates from the same root-tip show, besides the gigantic appendage on chromosome D, an additional, seventh, independent, miniature chromosome without visible constriction (Fig. 30a). In two other plates this miniature chromosome is absent (Fig. 30b), and in a fifth it is attached to C, but the large appendage is missing, and on both D-chromosomes there are entirely normal satellites (Fig. 30c). How the satellite was able to free itself from its fusion with the "appendage" is not clear.

The above-mentioned plates were taken from sections so distributed that the possibility of any kind of a chimera is excluded.

Somewhat similar to the aberration just described is the unique deviation characterizing two plants in the progeny of Plant 2536 (No. 14) and shown in Figures 31 and 32. At first glance it might be taken for a considerable lengthening of the short arm of A, but this "arm" proves to be always clearly disconnected from the other, whereas in a real A-chromosome the arms are closely connected. Moreover, on the long "arm" there may be usually quite plainly distinguished the typical head of a D-chromosome (Fig. 31). All this leads one to believe that in this case we have to do with a transfer of the short arm of A to the satellite of D. Then one of the supposed "D-chromosomes," always deprived of its satellite, will be in fact an A-chromosome having only a tiny remnant of its second arm. Such an interpretation leaves unexplained the excessive length of the translocated segment, which would impel one to suppose that there might be here involved another translocation too.

1) A similar phenomenon may have occurred also in the case of the previously described "inversion in D," since ARENKOVA (1939) observed in this plant pollen grains with a small D-chromosome minus its gigantic satellite.

These facts clearly demonstrate the possibility of origin of new chromosome types in result of a purely mechanical breack and with formation thereby of their new end surfaces (cf. MÜNTZING, 1934).

Despite all the above-described structural changes in the chromosomes, their number remained normal. As a result, however, of translocations in the meristematic tissue, there may subsequently occur at meiosis relations giving rise to gametes with an anomalous number of chromosomes. Those having one extra chromosome will be the most viable (cf. ARENKOVA, l.c.). Quite frequently such an extra chromosome proves to be a proximal segment of a D-chromosome involved in a translocation "from D to A." Due to its small size, it very often does not form a chiasma with its partner and remains in the form of a free univalent, uniting sometimes with a gamete containing a normal D-chromosome (cf. PETROV, 1935). As a result, in the progeny of aberrants with a translocation "from D to A" there are found individuals with an extra, seventh chromosome, a shortened D-chromosome. For example, in such an F_2 of ours 4 out of 82 plants had the indicated extra chromosome. Of analogous origin, apparently, is a similar aberration (col. 19), found by us in the progeny of the same plant (2461, No. 2), which gave three aberrants with a translocation "from D to A"—all the more, since in both cases the size of the shortened D-chromosome is the same (Fig. 33). Two other similar aberrations, originating from other plants (Nos. 14, 21), arose, apparently, from pollen of the same initial plant having an additional short D-chromosome.

A few combinations of chromosome changes were obtained: "from D to A" and C_{sh} (Fig. 7)—1; "from A to C" and D+x (Figs. 29 and 30)—2; A_{sh} and C_{sh}—1. They must be attributed, presumably, to the fusion of correspondingly different aberrant gametes.

We shall pass now to a *numerical analysis* of the different kinds of chromosome rearrangements. Such an analysis was made in our laboratory on the basis of nuclear plates in X-rayed seedlings (LEWITSKY and SIZOYA, 1934, 1935; SIZOYA, unpubl.). It is of interest to compare the numerical relations between the different types of aberrations in *separate cells* of X-rayed plants and in aberrant *individual*: arising from the latter. Most of the investigated translocations in X-rayed material were "from the long arm of A to the long arm of C". In the latest material of SIZOVA (unpubl.) this translocation was observed 55 times. Next in order were the translocations "from D to A" and "from D to C", observed 32 and 28 times, respectively. In our material *the same order* of frequency prevailed: 24, 5, and 3 times, respectively. It is worthy of note that in our material there occurred no translocations "from the long arm of one A-chromosome to the short arm of the other," found by SIZOVA 32 times. This may be explained only by the fact that a plant with such a translocation (A−a, A+a) could not produce balanced gametes.

The karyotypes of the gametes formed by our heterozygous translocants were subjected to special investigation in our laboratory (by means of pollen grains) by ARENKOVA (1939). This study showed that, in addition to the normal set (A, C, D) and balanced sets with translocations (e.g., A–a, C+a, D), there are formed some sets with the loss or addition of parts of chromosomes or much more rarely, whole chromosomes. As regards translocations between two homologues, there would then be obtained exclusively such only slightly viable gametes as, for instance, (1) A–a, C, D; and (2) A+a, C, D. The formation of a balanced zygote from two such mutually complementary gametes (in the case of the translocation spreading throughout the entire flower or inflorescence) is made difficult by the fact that *Crepis capillaris* is a cross-pollinated plant. There remains the possibility of the fusion of a normal gamete with a gamete having a deficiency or addition. Zygotes with a part of chromosome A missing were not once found by us, although, as we have mentioned, such pollen grains were encountered. Plants with extra whole A- and D-chromosomes have been reported by M. NAVASHIN (1926, 1929), and I found one such plant, as well as a C trisomic previously not encountered. In third-generation zygotes we observed extra parts of the D-chromosome, but plants with an extra part of an A-chromosome were not obtained even once, although the gametes necessary for such a combination (A, C+a, D) were formed in small number by plants with a heterozygous translocation "from A to C" (ARENKOVA, l.c.).

From all this it must, apparently, be concluded that the absence of such individuals which would be expected also as a result of the common translocation "from A to A" is due to a probable lack of balance in the distal segment of chromosome A. In this connection we wish to call attention to an aberration, presumably arising from a deviation similar to that just considered, viz., a translocation from one D-chromosome to the satellite of the other D-chromosome (Figs. 26–29). The occurrence of a plant with such an aberration shows that its formation encounters less difficulties than that of a plant with the translocation "from A to A" which we have just discussed. Undoubtedly such an aberrant was obtained by us in the form of a triploid, its origin being due presumably to an exceptional case of the formation of a diploid gamete with precisely this kind of translocation "from D to D". This remarkable translocation—previously not described—is shown in Figure 34.

Of the other aberrations reported by SIZOVA (l.c.) a considerable number (32) involve the *association* of two chromosomes. But this deviation, in the form of a complex of two chromosomes with two

constrictions, is incapable—as has been shown by the investigations of MATHER and STONE (1933)—of prolonged reproduction by somatic division, and, hence, cannot reach sexual elements.

As is seen from the foregoing, all the structural rearrangements of chromosomes obtained by us in the first generation were represented in a heterozygous state, *i.e.*, with an alteration of only one homologue—in one or two pairs. The next task consisted in obtaining these alterations in a homozygous state and in this way creating constant, in a cytological sense, new races with altered idiograms. With this aim in view the above-described heterozygous aberrants were either subjected to self-pollination or identical pairs were selected from the same family and cross-pollinated. In the case of one of the deviations ("from D to C"), due to failure of seeds to set when the flowers were bagged, it was found necessary to resort to open cross-pollination between two individual plants, but even so seeds were obtained from only one individual (2475/15). The same method was applied in several other cases. In this way there were obtained homozygous representatives of the following types (in the order of our foregoing exposition):

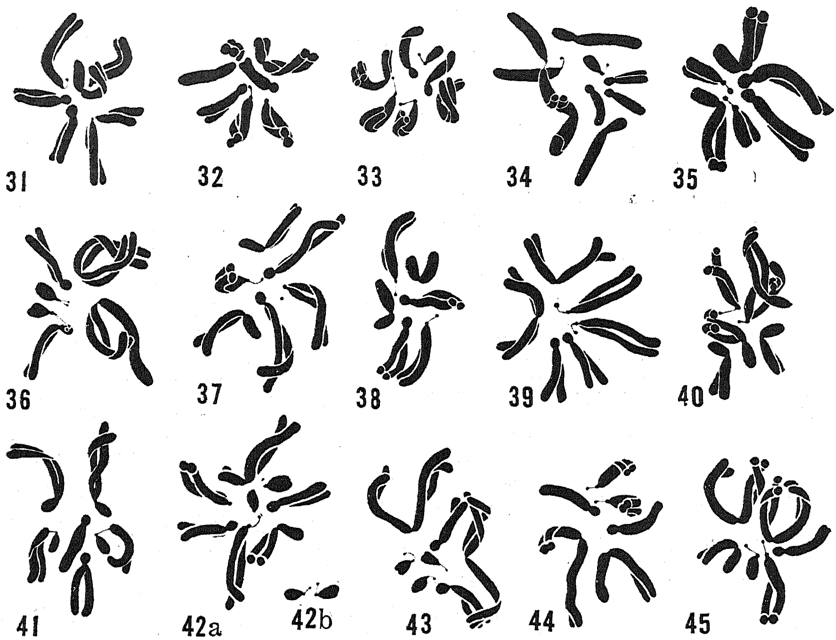
The translocation "from 2D to 2C" (Fig. 35) was obtained, by the method just described, in two individuals in the F_3 from the Family 2475/15 (cf. Fig. 2). The translocation "from 2D to 2A" (Fig. 36), in the form of the type with more markedly shortened D-chromosomes (initial Plant 2461), was obtained in four individuals in the F_2 by intercrossing F_1 plants, plates from which are shown in Figures 6 and 7.

The translocation "from 2A to 2C" (Fig. 37), in the form of the type with longer A-chromosomes, was obtained in two individuals of F_2 from an unbagged plant of the progeny 2533/23. Judging by the identicalness of the two A-chromosomes, there took place here self-pollination of the mother plant. An analogous type of the same translocation but with shorter A-chromosomes (Fig. 38) was also obtained in the F_2 from a plant left unbagged (2467/14). Its A-chromosomes, although similar, differed somewhat, so that complete homozygosity was not attained.

"Shift of the constriction" in 2A (Fig. 39) was obtained in five individuals in the F_2 from the Family 2501/20 (cf. F_1–Fig. 12) by self-pollination and from intercrossing in the Family 2501 (16×20).

"Shift of the constriction" in 2C (Fig. 40) was obtained in the F_2: (1) from pollination of 2472/17 (No. 10) by 2470/14 (No. 4) —however, there apparently took place self-pollination of the mother plant, since the homozygous aberrations obtained reproduce in both chromosomes the maternal type (F_1—Fig. 20); and (2) from self-

pollination of a plant (F_1—2461/3, No. 2) with a combined alteration, "from D to A" and "C_{sh}" (a plate from which is shown in Fig. 7), as a result of the combination of normal A- and D-chromosomes with altered C-chromosomes.



Figs. 31–45. 31, 32. Translocation from the short arm of A to the satellite of D. Two plates from two individuals (mother-plant 2536). 33. 7 chromosomes. Addit'on of a proximal portion of chromosome D. 34. Translocation from D to the satellite of another D in a triploid. 35–38. "Homozygous" translocations. 35. Translocation "from 2D to 2C." 36. Translocation "from 2D to 2A." 37. Translocation "from 2A to 2C" with longer A-chromosomes. 38. Translocation "from 2A to 2C" with shorter A-chromosomes. 39–41. "Homozygous" aberrations in one chromosome type. 39. "Constriction shifting" in 2A. 40. "Constriction shifting" in 2C. 41. Inversion in 2D. 42–45. Augmentative aberrations. 42. Tetrasomic in proximal portion of D. 43. 7 chromosomes. Addition of proximal portion D and translocation "from D to A." 44. One chromosome A with "constriction shifting," another with an addition of distal portion D. 45. Translocation "from D to A." Both A-chr. with an addition "from D, but only one D is correspondingly shortened. One of C-chr. with a "constriction shifting."

The translocation to the satellite in 2D (Fig. 41) was obtained in two individuals in the F_3 from the same initial plant (2467, No. 21) as the F_1 individuals represented in Figures 22–25.

Of the "augmentative" types there was obtained in a homozygous form only a "tetrasomic as regards the proximal portion of D" (Fig. 42a).[1] This solitary plant of this kind arose from crossing

1) In this plate the supernumerary (D–d) chromosomes do not look alike. In other plates from the same root-tip they appear as shown in Figure 42b.

two corresponding trisomics (Fig. 33), three of which were found in the F_2 progeny of an aberrant with both a translocation "from D to A" and a "shift of the constriction" in C (about which we mentioned above). Types with diverse supernumerary elements in their chromosome sets might, in general, be expected in the progeny of plants with translocations, in view of the formation by them of gametes with such elements. Thus, the pollen grains of the heterozygous aberrant with a translocation "from A to C" investigated by ARENKOVA (1939) included following karyotypes: 1) A C D—45.1%, 2) A−a C+a D—17%, 3) A−a C D—6.1%, 4) A C+a D—3.7%, 5) A A−a C D−5%, 6) A A−a C+a D—1.2%, 7) A C C+a D—11%. An aberrant with a translocation "from D to A" gave the following supernumerary elements D, D−d, d—in amounts from 1 to 21 per cent. It should be mentioned that the same aberrants gave pollen grain karyotypes with deficiencies ("from A to C":—a; "from D to A":—d), but, despite their considerable number (6% and 21%, respectively), plants with such deficiencies, even in a heterozygous state, were not found.

As regards expected "augmentative" types, in addition to all 3 types of trisomics—there occurred the following "partial trisomics":

First, a *trisomic as regards the proximal portion of* D (different from that described earlier—Fig. 33), which arose as a result of a translocation "from 2D to 2A" plus an extra shortened D-chromosome (Fig. 43); its formula 2(A+d), 3(D−d), 2C. This aberrant occurred in the progeny of a plant with a homozygous translocation "from D to A," as a result of the fusion of gametes (belonging to one plant or to two plants of the same type), one with a balanced chromosome set and one with an extra (D−d)-chromosome, an explanation of which we have already given above.

Since the proximal portion of D remaining after a translocation from the D-chromosome possesses the attachment constriction, it is added to the set in the form of a special, independent chromosome, increasing the total number to 7. The addition of an extra distal segment of the same chromosome, not having any attachment construction, can be realized only by the inclusion of the A-chromosome to which it was translocated. A simpler case of this kind is shown in Figure 44, where the D- and C-chromosomes are normal but one of the A-chromosomes has its short arm lengthened in a way typical for a translocation "from D to A," the other A-chromosome having only a "shift of the attachment constriction." In another case (Fig. 45) we have *two* A-chromosomes with such lengthened arms, but only *one* of the D-chromosomes has a correspondingly shortened arm (one of the C-chromosomes shows a "shift of the attachment constriction").

Both of these aberrants were revealed in the progeny of a double heterozygote with the formula: A_{sh}, (A+d), D, (D−d), C, C. In the first case the following gametes were encountered: 1) balanced: A_{sh}, D, C; 2) with a "duplication" of d: A+d, D, C. In the other case the gametes were: 1) balanced: A+D, D−d, C;[1] 2) with a "duplication" of d: A−d, D, C_{sh}.[2]

We have already mentioned the complete absence in our material of plants with karyotypes *lacking* some element of the normal diploid set. The only plant we found having a deficiency came from a triploid of a translocation "from D to A" (Fig. 46). This deficiency consisted in the absence of one of the small (D−d)-chromosomes (Fig. 47). Both plants arose in the progeny of a homozygous translocant—2(A+d), 2(D−d), 2C. Two combinations of gametes are possible for the formation of such a deficiency: either 2(A+d), 2(D−d), 2C plus (A+d), C; or 2(A+d), (D−d), 2C plus (A+d), (D−d), C. The occurrence of the first combination is very improbable, since "defective" gametes lacking a (D−d)-chromosome were not found in the material investigated by ARENKOVA (l.c.). In diploid gametes the absence of one of the (D−d)-chromosomes would, of course, be felt less, and, hence, the second alternative is the more probable.

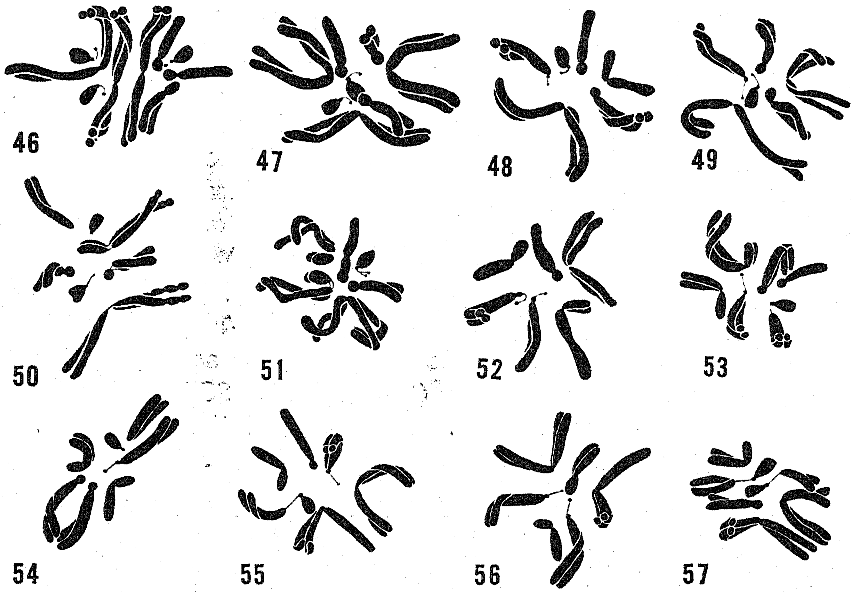
As a result of crossing the aberrants we have described we obtained a great diversity of different combinations, but, of course, almost exclusively in a heterozygous form. In many of them homologous chromosomes were altered in different ways, as, for example, "from D to A"+"from A to C" (Fig. 48), "from D to A"+A_{sh} (Fig. 49), "from D to A"+inversion in D (Fig. 50). A similar change shown in Figure 51 is accompanied by a "shift of the attachment constriction" in one of the C-chromosomes.

To secure a combination of homozygous aberrations from such plants is difficult, in view of the exceptional complications of meiosis. Therefore, for this purpose we employed the simpler changes affecting non-homologous chromosomes, such as "shift of the attachment constriction" in A and C (Fig. 52), translocation "from A to C" plus inversion in D (Fig. 53), and, lastly, inversion in D combined with a "shift of the attachment constriction" in A and C.

For the more speedy attainment of our aim we took for crossing plants already homozygous as regards one of the aberrations. In this direction we have so far made two attempts. The first was a cross between "from A to C"+D_{inv} and 2("from A to C"). As a result, there were obtained three plants with 2("from A to C")+D_{inv}

1) Or C_{sh}.
2) Or C.

(Fig. 54). The second cross was: $(A_{sh}+D_{inv}) \times (A_{sh}+2C_{sh})$. As a result, there were obtained two plants with a homozygous change in A and heterozygous changes in the two other chromosomes: $2A_{sh}$, D_{inv}, C_{sh} (Fig. 55). In their progeny we obtained, on the one hand, a homozygote—$2A_{sh}$, $2C_{sh}$, 2D (Fig. 56)—and, on the other, homozygous changes in the chromosomes A and D, combined with a heterozygous change in C, *i.e.*, $2A_{sh}$, $2D_{inv}$, C_{sh}, C (Fig. 57).



Figs. 46–57. **46, 47.** Triploid translocations "from D to A." **47.** The same and deficiency of one of diminished D-chromosomes. **48–53.** Double and triple aberrations. **48.** Translocation "from D to A" and "from A to C." All the six chromosomes are different. **49.** Translocation "from D to A" and "constriction shifting" in A. **50.** Translocation "from D to A" and "inversion" in D. **51.** Translocation "from D to A," inversion in D and constriction shifting in C. All the six chromosomes are different. **52.** Constriction shifting in A and C. **53.** Translocation "from A to C" and inversion in D. **54, 55.** Homozygous and heterozygous aberrations combined. **54.** Translocation "from 2A to 2C" and inversion D. **55.** "Constriction shifting" in 2A and C. Inversion in D. **56, 57.** Double homozygous aberrations. **56.** "Constriction shifting" in 2A and 2C. **57.** Inversion in both D, "constriction shifting" in 2A and C.

The diverse rearrangements of chromosomes described above are not reflected in any specific (to each of them) manner on the external morphology of the corresponding plants. It should be noted that *Crepis capillaris* is, in general, a very polymorphic species with an exceptional amount and diversity of intraspecific variations. The latter characteristic is undoubtedly still more intensified in the progeny of X-rayed plants. In addition to forms ordinarily occurring under natural conditions there appear quite often changes which

are specifically mutant in character, chiefly in the form of a general depression in growth and of abnormalities in development. Especially often the branches are more compact and thickly covered with narrow leaves. These changes occur, however, both among aberrants of different kinds and among karyotypically normal plants. It is possible that here, as in *Drosophila*, there takes place a specific reflection of definite structural chromosome changes on the phenotype, in the form of a so-called "position effect," but this could be established only by work with pure-line, homogeneous material, which in *Crepis capillaris*, a cross-pollinated plant, is difficult to realize.

The fertility of the investigated aberrants[1] varied widely.[2] In heterozygous translocants it was more or less markedly lowered, amounting in "from A to C" translocants to 50 per cent, in "from D to A" to 34 per cent, and in "from D to C" to less than 1 per cent. Of the two heterozygous inversions one (D_{inv}) showed only slightly lowered fertility (58 per cent) and the other (A_{sh}) almost normal fertility (67 per cent). Homozygous aberrants showed either close to normal ("from 2D to 2A"—67 per cent, $2D_{inv}$—77 per cent, $2A_{sh}$—61.5 per cent) or markedly lowered fertility, even as compared with the corresponding heterozygotes ("from 2A to 2C"—41 per cent, "from 2D to 2C"—0 per cent). The cause of the last-mentioned phenomena may be some kind of injury to the hereditary material of the plants, resulting from structural changes in certain chromosomes in the form either of lethal mutations or of a disadvantageous "position effect" (cf. DOBZHANSKY, 1930).

Markedly lowered fertility is a characteristic of plants with an extra proximal portion of D; addition of its distal portion (Figs. 44 and 45) or of its proximal portion in a double dose (Fig. 42) results in complete sterility. The last two types of aberrants are characterized in general by sharply lowered viability. Of three plants with a heterozygous addition of d, one was very frail and the other two died in the rosette stage. The tetrasomic "+2(D−d)" was also very frail. The triploid with a "D−d" deficiency (Fig. 47) showed exceptionally vigorous development and flowered profusely, but after producing only three seeds, died suddenly and without apparent cause.

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1) This problem has been investigated in greater detatil by Miss N. M. GRÜNER, from whose as yet unpublished data we have taken, in round numbers, our figures on fertility. For her investigation Miss GRÜNER took four plants of each type, spatially isolated from the other groups. From each plant ten heads were investigated. Fertility varied to a considerable extent from plant to plant and even from head to head of the same plant.

2) In the case of karyotypically normal individuals from one and the same progeny of X-rayed plants fertility averaged 69 per cent.

Some of the investigated F_2 plants died during the very early stages. Although it is possible that their death was due in part to purely external causes, since these plants had had their primary roots cut off, it was nevertheless interesting to compare the karyotypical peculiarities of these plants which had died with those of the plants which survived. The results of such a comparison are given in Table 2:

Table 2. Comparative viability of normal and aberrant F_2 plants

	Normal	Heterozygous aberrants	Homozygous aberrants	Aberrants with additional chromosome elements
Perished	8	10	3	4
Survived	76	135	14	8
%perished	10.5	7.4	21.4	50.0

Despite the paucity of data, they suffice to show clearly the negative effect on viability of homozygosity of aberrations and particularly of aberrations with extra chromosome elements.

In connection with the foregoing, it should be noted that in the progeny of intercrossed heterozygous aberrants there quite frequently occurred a markedly diminished number of aberrant plants, especially homozygotes, as compared with normal plants. Let us take, for example, the F_2 progeny of plants with a translocation "from D to A", this being one of the progenies regarding which we have comparatively more data. This F_2 arose partly from self-pollination of F_1 plants but chiefly from crosses between two karyotypically identical plants. Normal plants totaled 30, recovered heterozygotes—18, homozygotes—6, plants with an extra (D−d)-chromosome−5, plants with an extra d−1. These data may be explained in part by the relative numbers of various types of gametes formed by plants with this aberration. According to investigations of pollen grains in plants with a translocation "from D to A" (ARENKOVA, 1939), normal karyotypes (A, D, C) are greatly in excess of balanced translocations (A+d, D−d, C), giving heterozygous and homozygous aberrants. The considerable number of plants with an extra D−d-chromosome may be attributed to the comparative abundance of gametes with such an addition (20.8%), precisely in plants with this type of aberration. A certain rôle in the determination of the character of the progeny may also be taken by the different degree of viability of the pollen, which is highest in the normal karyotype, thereby determining the predominance of the latter in the competition in growth of pollen tubes, fertilization, and development of zygotes.

However that may be, once aberrant karyotypes have arisen—even when surrounded by normal plants—they are reproduced in the progeny, although in the case of different aberrants, apparently, to a very different extent. This may be illustrated by data on the progeny of aberrants grown side by side with normal plants,[1] such as given in Table 3:

Table 3. Progeny of aberrants grown side by side with normal plants

Progenies \ Parents	Heterozygous				Total from heterozygous aberrants
	"from D to A"	"from A to C"	D_{inv}	A_{sh}	
Homozygous aberrants	2	—	2	—	4
Heterozygous aberrants	2	4	1	2	8
Normal	3	3	1	—	7
Other misc. aberrants	2	—	1	—	3
Progenies \ Parents	**Homozygous**				**Total from homozygous aberrants**
	2("from D to A")	2("from A to C")	$2D_{inv}$	$2A_{sh}$	
Homozygous aberrants	4	5	7	—	16
Heterozygous aberrants	5	11	—	10	26
Normal	—	—	—	—	—
Other misc. aberrants	3	—	2	—	5

Despite the small amount of data at our disposal, nevertheless to a certain extent they mutually check one another in the progenies of corresponding heterozygous and homozygous individuals. Reproduced to the greatest extent was the "inversion in D"—from homozygous parents even 100 per cent; to the smallest extent, the "shift of the attachment constriction" in A—in the homozygous state not at all. The translocations "from D to A" and "from A to C" occupy an intermediate position, with a clear preponderance of the former, both from heterozygous and homozygous parental forms.

In connection with the data here set forth the question was raised as to the types of gametes formed by the structural aberrants studied by us. For this purpose first metaphases of pollen grains were investigated (ARENKOVA, 1939). The results obtained are given in Table 4. Direct cytological investigation reveals a far greater diversity of karyotypes in gametes formed by heterozygous translocants than may be supposed on the basis of pollen sterility and the karyotypes of the progeny (BURNHAM, 1930). In addition to I) normal, II) balanced aberrant, and two types of unbalanced aberrant karyotypes: with a deficiency (VI, VII–4) or a duplication

1) The order in which the plants were grown was: normal, aberrant, aberrant, normal. The assured equal probability of pollination between the aberrants themselves and of pollination by normal plants.

(V, VI, VII–3), there are formed a number of karyotypes with an extra chromosome: smaller than normal (V–5, VI–6, VII–6), normal (V–4, VI–5, VII–5), and even larger than normal (VII–7, 8). The number of such pollen grains sometimes amounts to as much as 11

Table 4. Karyotypes of pollen grains formed by structural aberrants of *Crepis capillaris* (ARENKOVA, 1939)

Somatic karyotypes	Pollen fertility in %	No. of investigated plates	Karyotypes of pollen grains and their relative (%) numbers	
I AA CC DD	100	50	1. ACD	100
II A+d A+d CC D−d D−d	100	50	1. A+d C D−d	100
III AA$_{sh}$ CC DD	90	44	1. ACD	50
			2. A$_{sh}$ CD	48
			3. AA$_{sh}$ CC DD	2
IV AA CC DD$_{inv.}$	80	96	1. ACD	51
			2. ACD$_{inv.}$	44.8
			3. ACD D−d	2.1
			3a. ACd	—
			4. AA CC DD$_{inv.}$	2.1
V A A+d CCD D−d	47	251	1. ACD	44
			2. A+d C D−d	27.8
			3. A+d CD	20.6
			3a. AC D−d	—
			4. A+d C D−d D	1.6
			4a. AC	—
			5. ACD D−d	5.6
			5a. A+d C	—
			6. A A+d CCD D−d	0.4
VI A$_{sh}$ A+d CC D D−d	25	72	1. A$_{sh}$ CD	33.4
			2. A+d C D−d	16.7
			3 A+d CD	5.5
			4. A$_{sh}$C D−d	20.8
			5. A+d C D−d D	1.4
			5a. A$_{sh}$ C	
			6. A$_{sh}$ C D D−d	20.8
			6a. A+d C	—
			7. A$_{sh}$A+d CCD D−d	1.4
VII A A−a C C+a DD	47	82	1. ACD	45.1
			2. A−a C+a D	17
			3. A C+a D	3.7
			4. A−a CD	6.1
			5. A A−a C+a D	1.2
			5a. CD	—
			6. A A−a CD	5
			6a. C+a D	—
			7. AC C+a D	11
			7a. A−a D	—
			8. A−a C C+a D	8.5
			8a. AD	—
			9. A A−a C C+a DD	2.4

or even 21 per cent, exceeding in the latter case (VI–6) even the balanced karyotype (VI–2). Of all these types only duplications of D–d and d occurred frequently in the progeny of our heterozygous translocants.

The fertility of the pollen of our heterozygous translocants is near to the % number of normal karyotypes. Balanced aberrant karyotypes are observed in considerably less number already at the first division in pollen grains; as the latter further mature the relative number of such karyotypes probably continues to decrease. This explains a lowered number of homozygous translocants in the progeny of heterozygous translocants. Hence, the decrease in viability of structurally changed chromosomes, in the gametophyte, i.e. in the haploid state is very markedly in evidence.

Marked differences are observed in the relative number—and, consequently, in the viability—of duplications from different chromosomes. First place is taken by chromosome D, the number of duplications of whose distal segment (d) may reach 20.6 per cent (V–3); second comes chromosome C, with 8.5 per cent (VII–8), and in combination with "a" even 11 per cent (VII–7). The least viable, apparently, are duplications from chromosome A: +A—1.2 per cent (VII–5), +(A–a)—5 per cent (VII–6), +a—3.7 per cent (VII–3). This makes understandable the complete absence of any aberrants with duplications in the progeny of plants with a translocation "from A to C", while we found ten such aberrants with a duplication of the distal segment of D (5 in F_1 and 5 in F_2 and F_4; cf. Figs. 44 and 45). It is of interest that unreduced pollen grains are formed by structural heterozygotes, while they do not occur in the case of structural homozygotes or normal plants.

Discussion

The above-surveyed rearrangements of the karyotype based on a redistribution of chromosome portions arise not only as an effect of X-ray treatment. The first genetically established translocations in *Drosophila* (cf. MORGAN, BRIDGES, and STURTEVANT, 1925), as well as a considerable number of similar architectural rearrangements of chomosomes in *Datura* and Maize, arose without any special outside agencies, as has also been reported for *Crepis* (NAVASHIN, 1926, 1931).

An accelerated frequency of occurrence of the same chromosome changes was also established as a result of the aging of seed (NAVASHIN, 1933; NAVASHIN and GERASSIMOVA, 1936) and of such natural factors as high temperature combined with a moist environment (NAVASHIN and SHKVARNIKOV, 1933; KIRNOSOVA, 1935; PETO, 1935). In the progeny of *Crepis tectorum* plants grown from old seeds there were obtained two aberrants similar to those described by us, with chromosome changes both of a heterozygous and homozygous type (GERASSIMOVA, 1935).

A potent and wide spread source of "spontaneous" chromosome changes seems to be also a genotypical control of chromosome stability (DARLINGTON, 1937; LEWITSKY, 1937).

In addition to reported cases of the occurrence of structural chromosome changes in the progeny of cytologically (or genetically) studied individual plants, they have been revealed in a number of species (*Campanula, Aucuba, Tradescantia, Rhoeo, Briza, Polemonium, Pisum, Zea*) in separate individuals, clones, and strains (cnf. MÜNTZING, 1939).

In some cases structural chromosome differences distinguish geographical races and species (*Drosophila*,[1] *Datura*[2]). Such cytological peculiarities are, as is well known, especially widespread among species of the genus *Oenothera* (cf. CLELAND, 1936) where, as a result of reciprocal translocations, there is set up a unique cytogenetic mechanism of meiosis and heredity.

Conclusions as to chromosome differences between various races or species on the basis of translocations (chiefly reciprocal) are usually drawn—as in the case of the genera just mentioned—from the formation by diploid types of multiple chromosome configurations at meiosis instead of the ordinary pairs. In some cases conclusions as to such regroupings of portions of the chromosome set may be drawn on the basis of differences in the morphology of the chromosomes of the respective races. Such an interpretation is made, for example, by SVESHNIKOVA (1936) in the case of karyotypical differences between *Vicia sativa* and *V. amphicarpa*, as well as between two karyotypical races of *V. angustifolia* reported by SVESHNIKOVA (1929). Grounds for this lie in the possibility of the formation of "tetrapartite" chains by corresponding chromosomes (SVESHNIKOVA, 1936). In other cases conclusions may be drawn about definite structural rearrangements of chromosomes by analogy with artificially induced aberrations, as in the cases of *Crepis pulchra* (HOLLINGSHEAD and BABCOCK, 1930), *Rumex acetosa* (YAMAMOTO, 1933) and *Paris hexaphilla* (HAGA, 1937).

The chains of chromosomes found by KIHARA (1932) at meiosis in the intergeneric hybrid *Aegilops speltoides*×*Triticum monococcum* indicate that there are structural chromosome differences between these species, and led KIHARA to conclude that these differences are of fundamental significance as regards the difficulties of chromosome conjugation in their hybrids, resulting in their sterility and, consequently, in the sexual isolation of the respective forms.

The same conclusion is drawn from the figures of "bridges" in

1) DOBZHANSKY, 1937.
2) BLAKESLEE, 1932, BLAKESLEE et alii 1937.

the anaphase of heterotypic division, which appear sometimes in species and race hybrids (MÜNTZING, 1934; RICHARDSON, 1936; DARLINGTON and GAIRDNER, 1937), as well as in pure species (UPCOTT, 1937; SAX, 1937; STEBBINS, 1938; GEITLER, 1937).

It is very probable that the same reasons lie at the basis of the partial non-conjugation of chromosomes in race hybrids described in wheat (SAPIEGUINE, 1927; HOLLINGSHEAD, 1932) and *Galeopsis* (MÜNTZING, 1938). Thus, structural chromosome rearrangements may constitute an essential element in evolution of taxonomic separation (cnf. DOBZHANSKY, 1937).

Especially striking illustrations of this are given by material where it is possible to see the detailed inner architecture of the chromosomes in the form of so-called "disks," which are perhaps identical with the loci of definite genes. In the way of first results there may be noted the cytological proof of a genetically established inversion in chromosome III of *Drosophila simulans*, as compared with *D. melanogaster* (PÄTAU, 1935), differences based on cytologically established inversions in races of *D. pseudoobscura* (DOBZHANSKY and STURTEVANT, 1938) and, lastly, the new species, *D. miranda*, differing from *D. pseudoobscura* in a number of definite translocations and inversions (DOBZHANSKY & TAN, 1936). Of great significance also are the cytologically established repetitions or "duplications" of definite groups of "disks" in *D. melanogaster* chromosomes (BRIDGES, 1935).

All the data here set forth—particularly the complete correspondence between race and species differences in chromosome architecture in nature and experimentally induced hereditary chromosome rearrangements—leave no doubt as to the evolutionary significance of such cytological changes.

Summary

1. Out of a total of 491 investigated plants in the progeny of *Crepis capillaris* X-rayed during the seedling stage 84, or 17.1 per cent, proved to deviate from the normal as regards their karyotype. Most of these aberrants (74) were characterized by structural chromosome changes. The data are summarized in Table 1 (p. 2).

2. In a number of cases several individuals with identically altered chromosomes arose from one mother plant, indicating their origin from a single aberrant cell of the latter, which had given rise to an entire aberrant sector or part of the mother plant.

3. The most common types of visible cytological changes were translocations, shown by the shortening of one chromosome and the

corresponding lengthening of another, and inversions, resulting in alterations in the morphology of the chromosomes concerned.

4. All of these changes were first obtained in a heterozygous state, and afterwards produced in a homozygous state.

5. In addition to balanced changes, cases of duplication were also obtained, both of whole choromosomes (all three types) and of separate parts of chromosome D, distal (of various kinds) and proximal. The latter change was also obtained in the form of a tetrasomic.

6. Some of the investigated structural aberrants were characterized by lowered fertility. In most cases this decrease in fertility was more marked in the heterozygous state; in others—in the homozygous state. In the former this was due, presumably, to cytological reasons; in the latter—to genetical, perhaps to a lethal expression of the "position effect."

7. In the progeny of structural heterozygotes structurally altered homozygotes occur, as a rule, less frequently than normal plants.

8. Among the karyotypes of pollen grains formed by structurally heterozygous aberrants normal karyotypes are, as a rule, considerably more numerous than balanced aberrant karyotypes. In two of the investigated heterozygous translocants there were formed a considerable number of pollen grains with karyotypes having an additional chromosome or part of a chromosome. The total diversity of chromosome sets of the male gametophyte reached as many as nine types.

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Cytological Studies on Yeast Cells with Special Reference to the Budding Process[1)]

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The method of nuclear division in budding yeast cells has long been a subject of discussion and controversy. Wager (1898), Guilliermond (1904; 1910; 1919), Wager and Peniston (1910) and others have described a typical amitotic type of division, while Swellengrebel (1905), Fuhrmann (1906) and Kater (1927) describe the nuclear division as a mitotic process or some variation of it. The smallness of the yeast cell, together with its obscuring cellular inclusions, makes cytological studies extremely difficult and interpretations in many instances questionable.

We have studied the division process of budding yeast by certain of the methods employed by previous investigators and, in addition, have utilized the Feulgen technique in an effort to throw further light on this problem. Accordingly, it is the purpose of this paper to report the results of our studies in the event that they may add to the general knowledge of this subject.

No effort has been made here to review all the literature relating to the numerous studies on the budding process of yeast, since good summaries of the literature have been given by Wager (1898), Wager and Peniston (1910), Guilliermond (1910; 1919) and Kater (1940).

Materials and Methods

The material used in these experiments was a pure strain of *Saccharomyces cerevisiae*.[2)] The stock was kept in a sterilized flask in a refrigerator at 5°C. and when taken from the refrigerator was practically in a non-budding condition.

A small portion of the compressed yeast (a piece approximately the size of a pea) was placed in a test tube containing about 20 cc. of Williams' media (Williams, 1920) and then placed in an incubator

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2) It is indeed a pleasure to express here our indebtedness to the authorities of the Anheuser-Busch Co. for their kindness in supplying us with the yeast.

at 30°C. until active budding occurred. Permanent slide preparations were made by coating a clean slide with egg albumin, placing on it a few drops of a concentration of the cells obtained by centrifuging with an electric centrifuge, and evaporating the excess media. However, the slides were never allowed to become completely dry before they were fixed. The following fixatives were used: Bouin's, Schaudinn's, and saturated mercuric chloride in 2% acetic acid. In most instances cells fixed in Bouin's solution were stained in Heidenhain's haematoxylin and those fixed in Schaudinn's solution were stained in 5% methylene blue. The Feulgen reaction was employed following mercuric chloride-acetic acid and Schaudinn's fixation. The Feulgen reaction and technique used are those described by Margolena (1932) and Ludford (1928).

The yeast cells were also cultured in a mixture containing 2% colchicine in Williams' media. They were subsequently fixed and stained in the same manner as the untreated cells mentioned above.

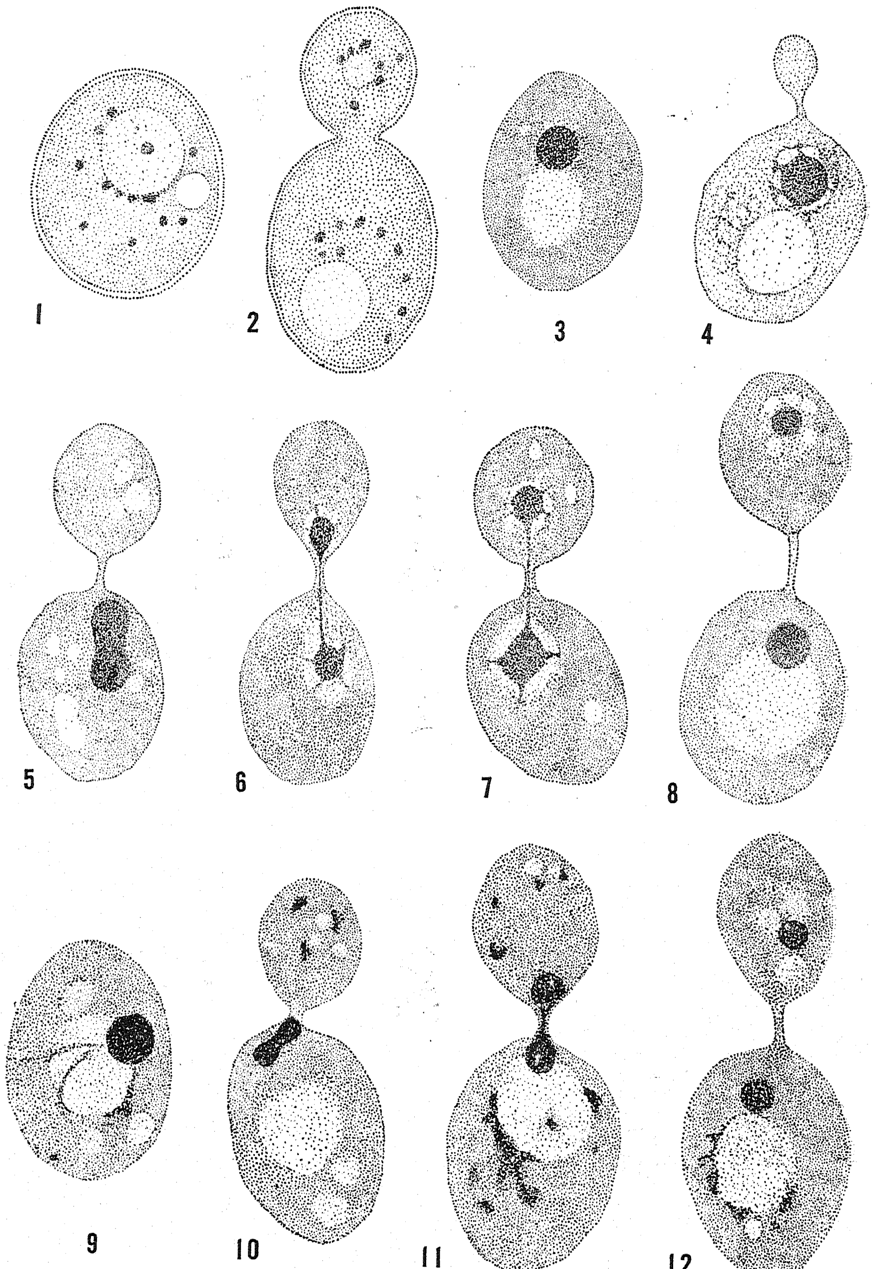
Description

1. The living unstained cells.

A study of the living unstained yeast cell, even under the oil immersion lens, reveals comparatively little of its internal structure (figs. 1, 2). The cells of *Saccharomyces cerevisiae* are round to slightly oval in shape and vary in size from 5 to 10 micra. The mother cell is frequently somewhat elongated during the process of budding (fig. 2). The cytoplasm is translucent and usually contains one (occasionally several) large vacuoles, one or more smaller vacuoles probably containing glycogen (Wager and Peniston, 1910), and a varying number of small highly refractive granules (figs. 1, 2). The granules exhibit Brownian movement and stain intensely with methylene blue. They are in all probability the volutin granules described by Wager and Peniston (1910), Guilliermond (1919), and others. Studies of the living cells reveal no structure that we could unquestionably interpret as the nucleus. Likewise, studies of the budding cells disclosed no clue as to the mode of nuclear division during that process. For a more complete discussion and description of the normal yeast cell see Wager (1898) and Guilliermond (1919).

2. Budding cells of stained preparations.

Since studies of the living cells reveal very little in regard to their nuclear division and other intricate cellular phenomena, it was necessary to employ various staining techniques. In addition, it was found desirable to use material from fresh cultures, since the accumu-



Figs. 1–12. 1. Living, unstained cell, showing thin cell wall, refractive granules, large central vacuole containing granules, small glycogen vacuole, and translucent cytoplasm. 2. Living, unstained cell with well developed bud. 3. Normal cell, fixed in 2% acetic acid saturated with mercuric chloride and stained by the Feulgen technique, showing nucleus, and large vacule. 4–8. Cells in the process of budding. Fixed in saturated mercuric chloride in 2% acetic acid, stained by the Feulgen

lation of glycogen and granules with a high affinity for the commonly used nuclear stains in old cultures tends to obscure the nucleus and makes interpretation of nuclear division very difficult. Different structures have been described as the nucleus by those who have worked on yeast and, because of this fact, the Feulgen reaction which is considered relatively specific for the nuclear chromatin of yeast by Margolena (1932) was employed. Cells stained according to the Feulgen technique consistently revealed a definite rounded or irregular shaped body in the cell which is pink or light red in color (fig. 3). Heidenhain's haematoxylin preparations demonstrated a comparable structure which here appears as a dark body against a light background (fig. 9). This body we have interpreted as the nucleus for the following reasons: (1) a similar structure stained in Heidenhain's haematoxylin has been described as the nucleus by certain other workers (Guilliermond, 1919); (2) Margolena (1932) has found the Feulgen reaction to stain the nucleus of yeast cells although Feulgen (1924) and Westbrook (1930) report a negative reaction for the nucleus of yeast and bacteria (cf. Margolena, 1932); (3) no other structure was found which could possibly be interpreted as a nucleus apart from that stained by the Feulgen reaction; and (4) this body (nucleus) undergoes a typical amitotic division during the budding process.

In many cells that were stained in Heidenhain's haematoxylin the nucleus seems to be associated with a vacuole-like structure, and it is difficult to determine whether this appearance is due to shrinkage or to the presence of an actual vacuole (figs. 4, 6 and 7). All the fixed cells show some shrinkage.

The history of the nucleus during the budding process is illustrated in figures 4, 5, 6, 7, 8, 10, 11, and 12. Here it will be observed that the division of the nucleus constitutes a typical amitotic process, comparable in many respects to the conditions described by Guilliermond (1904; 1919), and others. In addition to the above mentioned cytological evidence, experiments with colchicine, which is known to inhibit mitosis (Ludford, 1936; Beams and King, 1938; and others) revealed that the budding process of the yeast cells was not noticeably affected by its action. A cytological study of the cells treated with colchicine shows a typical amitotic division of the nucleus. Furthermore, it has been shown by Richards (1938) that colchicine actually stimulates budding rather than inhibits it.

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technique, showing nucleus in various stages of division. **9.** Normal cell, fixed in Bouin's solution, stained in Heidenhain's haematoxylin, showing nucleus, one large vacuole, three small glycogen vacuoles, and dark staining granules. **10–12.** Normal cells in the process of budding. Fixed in Bouin's solution stained in Heidenhain's haematoxylin.

Discussion

As previously pointed out, the size of the yeast cells, together with the difficulties in interpreting their stained cellular contents, has made the problem of nuclear division a subject of considerable controversy. Wager (1898), Wager and Peniston (1910), Guilliermond (1919), and Kater (1927) have all described the nucleus and nuclear division. The fact that no stain specific for nuclear materials was available probably accounts for a part of the difference in interpretations given by the various workers on yeast. Inasmuch as the Feulgen reaction has been applied by Margolena (1932) to the nucleus of certain botanical material with positive results, it seemed worthy of consideration in any attempted solution of the problem of nuclear division in yeast. Since we too have found that the Feulgen method stains the nuclei of yeast cells, we agree with Margolena (1932) who, while commenting upon the reaction of the Feulgen method with yeast nuclei, states: "At present it suffices to say that either the yeast cells contain some of the same kind of chromatin as do nuclei of other organisms, or else Feulgen's reaction is not so specific for one particular kind of nucleic acid as claimed by its author." The former suggestion now seems correct since Hillary (1939) states that Feulgen, Behrens, and Mahdihassan have developed a method of separating a pure plant nucleic acid from plant nuclei. "Thus plant and animal nucleic acids are one and the same thing and the nucleal reaction works equally well with both" (Hillary, 1939).

The fact that one group of workers (Guilliermond, 1904; 1910; 1919; Wager and Peniston, 1910) believes that the division of the nucleus during the budding process is amitotic while another group (Swellengrebel, 1905; Fuhrmann, 1906; Kater, 1927) is of the opinion that the division is mitotic necessitates consideration of the division phenomena. The discovery of mitosis and the recognition of its general occurrence in the plant and animal kingdoms led many workers to doubt that amitosis ever occurs as a normal process. Some authors were of the opinion it occurred only in pathological and degenerating cells. However, in the words of Conklin (1917), "The number of cases in which nuclear constriction is known to occur regularly in certain tissue cells is too great to warrant the belief that it is always a pathological phenomenon." Nevertheless, it seems that some writers were ready to reject amitosis and set up a defense in favor of mitotic division merely to be in harmony with modern genetic reasoning. The work of Kater (1927) seems to place itself in this category. That he was not able to establish conclusively

the process of mitosis in the budding yeast cells was evident when he said, "The above account leaves two points without adequate solution, namely the transformation of the nucleus into chromosomes and the migration of chromosomes through the isthmus." Several recent papers have appeared which show that amitosis occurs as a normal process in the division of the nucleus and cells of certain tissues of higher animals (see for instance Bast, 1921). Furthermore, the work of Richards (1938) which we have confirmed here, namely that colchicine does not inhibit the budding process in yeast, seems to lend strong support to the view that the budding mechanism does not take place by means of a mitotic process. This is substantiated by the fact that colchicine readily destroys the spindle and blocks the division of the cytoplasm in both plant and animal cells where mitosis is normally known to occur. That the colchicine actually enters the yeast cells was established by Richards (1938) who found that it stimulated the growth process rather than inhibited it. In addition, King and Beams (1940) have found that fission of certain protozoa occurs in strong colchicine solutions as also does ameboid movement. In these protozoa too no apparent evidence of a mitotic spindle has been found.

Accordingly, for the present at least, it seems that the majority of the evidence supports the view that the division process in budding yeast is amitotic and until better methods are developed for studying this process we believe that it should be considered as such.

Conclusions

1. The cells of the yeast, *Saccharomyces cerevisiae,* possess a definite compact nucleus which gives a positive reaction with the Feulgen method.
2. All cytological evidence shows that the division of the yeast cells during the budding process is amitotic.
3. Colchicine in strong concentrations does not inhibit the budding process in yeast. This is in striking contrast to its action upon other plant and animal cells which are known to divide by mitosis.

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Studies in the Scrophulariaceae: I. The cytology of *Angelonia grandiflora* C. Morr. and some related genera

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I. Introduction

Previous work on the cytology of the Scrophulariaceae is comparatively meagre and that too is confined to those members of this family, which grow in the temperate regions. Warren (1924) has described hybrids obtained between different species of *Digitalis*. Håkansson (1926) has described the cytology of *Celsia* and *Verbascum*. Buxton and Newton (1928) gave the chromosome numbers of a number of plants of this family and have also described interspecific crosses in *Digitalis*. Lawrence (1930) found a regular polyploid series in the chromosome numbers of the genus *Verbascum* and regarded eight as the probable basic chromosome number of the genus *Verbascum* and also of many other members of this natural order. Wanscher (1934) has discussed the question of the basic chromosome number of the family Scrophulariaceae and suggests four as the primary number. Practically no work of importance

has been done on the cytology of South Indian representatives of this family and the chromosome numbers of most of them are not known. Srinath (1934) found the haploid chromosome number of *Herpestis monniera* to be twenty-four. Krishna Iyengar (1937) observed eighteen bivalents during the heterotypic division of the megaspore mother cell in *Sopubia delphinifolia*. In another paper published elsewhere, the development of the female gametophyte, the embryo and the endosperm in some genera of the Scrophulariaceae has been described. In the present paper, the chromosome numbers of a number of South Indian genera have been determined for the first time. Besides, the cytology of *Angelonia grandiflora*, a South American species, which is grown here extensively as a garden plant on account of the beautiful flowers which are present throughout the year, is also described.

II. Material and Methods

Materials of *Angelonia, Stemodia, Russelia,* and *Scoparia* were obtained from plants grown in the University Botanical Gardens, Annamalainagar. *Dopatrium* was collected from the neighbouring rice fields, where it grows in profusion, while materials of *Vandellia* were collected from the marshy areas round about Annamalainagar. Root-tips were fixed at about 9–30 A.M. when they were found to be in active division, in chrom-acetic formalin of Karpechenko and Langlet (Manton, 1932) without prefixation in Carnoy. Anthers of the required stages of development were determined by the aceto-carmine smear method and fixed in chrom-acetic formalin. In the case of *Dopatrium*, the anthers could not be fixed individually, on account of the fact, that the two fertile anthers present are very small and so whole buds had to be fixed. In order to facilitate proper penetration, buds were pre-fixed in Carnoy's fluid, before fixation in chrom-acetic formalin. Very young buds were fixed in formalin-acetic alcohol, for studying the development of the microsporangium. Materials were dehydrated in alcohol, cleared in chloroform and embedded in paraffin. Sections were cut at a thickness varying from 6 to 12 microns. Heidenhain's iron alum haematoxylin and Newton's iodine-gentian violet were the stains used.

III. Observations

(1) *Angelonia grandiflora* C. Morr.

The development of the anther. Unlike many other members of this family, where reduction in the number of anthers is common,

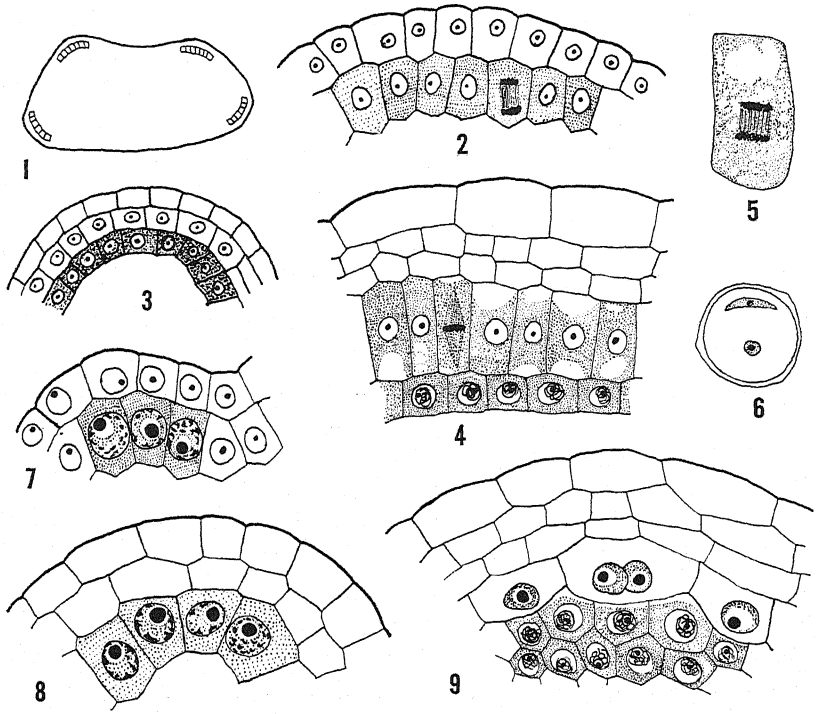
in *Angelonia,* all the four anthers are fertile. Since the chromosomes are very small, the prophase of the meiotic division could not be studied.

The primary archesporium of the anther is differentiated soon after the anther appears four-lobed in cross-section. The primary archesporium consists of four bands of hypodermal cells, one in each lobe (Fig. 1). Each band consists of about six to eight cells in cross-section (Fig. 2). Such extensive archesporia have been reported in *Digera arvensis* (Joshi and Rao, 1934), *Portulaca oleracea* (Cooper, 1935) and *Orobanche aegyptiaca* (Srivastava, 1939). The archesporial cell can be easily distinguished from the remaining cells by their larger size, larger nucleus, and richer cytoplasm. The anther lobes become more and more prominent after the differentiation of the primary archesporium.

The primary archesporial cells divide periclinally, giving rise to a layer of primary wall cells, or parietal cells to the outside and a layer of primary sporogenous cells to the inside (Fig. 3). In Fig. 2 one of the archesporial cells is in the telophase of this division. The primary wall-cells divide periclinally repeatedly giving rise to three layers of wall-cells of which the innermost functions as the tapetum (Fig. 4). The layer of wall-cells immediately outside the tapetal cells get elongated tangentially and is finally crushed due to the radial increase in size of the tapetal cells.

The tapetal cells enlarge and stretch out radially (Fig. 4). They have to start with a single prominent nucleus and deeply staining cytoplasm. The single nucleus of the tapetal cells undergoes a division, just at the time, when the pollen mother-cells are in late prophase (Fig. 4), and they remain bi-nucleate throughout. This division of the tapetal cells is by ordinary mitosis. Figs. 4 and 5 show different stages in the mitotic division of the nucleus of the tapetal cells. Some workers have reported fragmentation or amitosis as the typical method of nuclear division of the tapetal cells. For instance, O'Neal (1920) found that the nuclei of the tapetal cells divide by amitosis in *Datura stramonium.* Subsequently, however, Bonnet (1912) in a critical study of the tapetal cells of ten species of Angiosperms, found that the division of the tapetal cells was by ordinary mitosis. Cooper (1933) also found the tapetal cells dividing by ordinary mitosis in 35 species of Angiosperms, and he has classified the 43 species, which he has studied, into three groups, according to the behaviour of the tapetal nucleus; (1) in which the tapetal cell continues to be uni-nucleate without division, (2) in which the nucleus divides once and remains bi-nucleate and (3) in which pluri-nucleate tapetal cells are present.

Large vacuoles are a characteristic feature of the tapetal cells. Joshi (1936) reports the entire absence of vacuoles in the tapetum of *Stellaria media*. The tapetum in the later stages disorganises and finally disintegrates. The pollen mother-cells undergo a period of rest, during which time, they increase in size. In the early stages, the mother cells remain packed together closely. They begin to round off at about the stage of diakinesis.



Figs. 1–9. (Figs. 1-5 *Angelonia grandiflora* and Figs. 6-9 *Stemodia viscosa*). 1. The origin of the archesporium of anther as four bands of hypodermal cells below each corner of the anther sac. ×145. 2. One of the archesporial cells dividing to cut off a layer of primary parietal cells. ×1515. Fig. 3 shows an inner layer of primary sporogenous cells (shaded) and an outer layer of primary wall cells. ×420. Fig. 4 shows three layers of wall cells, the innermost functioning as the tapetum. One of the tapetal nuclei is in the metaphase of mitotic division. ×1515. 5. A tapetal cell, the nucleus of which is in the telophase of mitotic division. ×1515. 6. A bi-nucleate microspore at the time of shedding. ×1515. 7. A band of primary archesporium. ×1515. Fig. 8 shows an inner sporogenous layer and an outer parietal layer. ×1515. Fig. 9 shows three layers of wall cells, the innermost of which is the tapetum. ×1515.

Diakinesis. At early diakinesis, the twenty chromosomes, which commonly form ten pairs, are distributed along the periphery of the nucleus (Figs. 10 and 11). The nucleolus also has a rather peripheral position (Fig. 10). All the pairs are approximately

equidistant from one another. The equidistant spacing of the bivalents according to Lawrence is due to a repulsion phase, which begins at early diakinesis and continues until mid-diakinesis and the equal spacing of the bivalents, suggests that the inter-bivalent repulsion is also equal. At late diakinesis, some of the bivalents come closer together and this may be due to a weakening of the force of repulsion. This weakening of the force of repulsion continues until at prometaphase, all the bivalents are in close assemblage in the centre of the nucleus. Catcheside (1937) found that the bivalents secondarily paired at the end of prometaphase, are those that have occupied neighbouring positions at diakinesis. With the coming of metaphase, the inter-bivalent repulsion, which reaches its minimum at prometaphase again increases and as a result, the closely assembled group of bivalents of the prometaphase stage are spread out into a level plate. The two members of a bivalent are connected to one another in either of two ways, by one end only or at both ends (Fig. 10). Ring bivalents are quite common. At late diakinesis (Fig. 14) the chromosomes of each bivalent come to lie closer together and the pollen mother cell is bigger than in the earlier stage. Occasionally, trivalents and quadrivalents were to be found (Figs. 12 & 13).

Prometaphase. The converging movement of the bivalents, which begins at mid-diakinesis, proceeds, until the bivalents are in close assemblage in the centre of the nucleus. The nucleolus disappears (Fig. 15). No indication of secondary association of chromosomes, which manifests itself in a prominent manner in the succeeding metaphase is to be found in this stage.

Metaphase. Metaphase follows prometaphase. The ten bivalents are arranged on the equatorial plate (Fig. 16). The characteristic feature of metaphase is the association or approximation of the separate bivalents (Figs. 17 to 23). This secondary association is prevalent in the majority of pollen mother cells. The paired bivalents are similar in size and configuration. A variable number of bivalents are seen to be secondarily associated. Figures 16 to 23 show a number of metaphase I plates arranged in the order of their secondary association, of which Fig. 16 shows the stable configuration, which consists of two bivalents in the centre, with the remaining eight arranged in a ring around it. This pairing is exhibited very clearly in the first metaphase, first anaphase, and second metaphase. Table 1 gives a summary of the various types of secondary association.

In computing the number of secondary associations in each case, an association of three bivalents is counted as two, and that

of two bivalents as one. The number of secondary association per plate ranges from one to five, the mode being two. The maximum of five associations was seen twice in 31 plates examined. It shows one group of three bivalents, three groups of two bivalents each and a single bivalent, on the whole making five separate associations (Fig. 23).

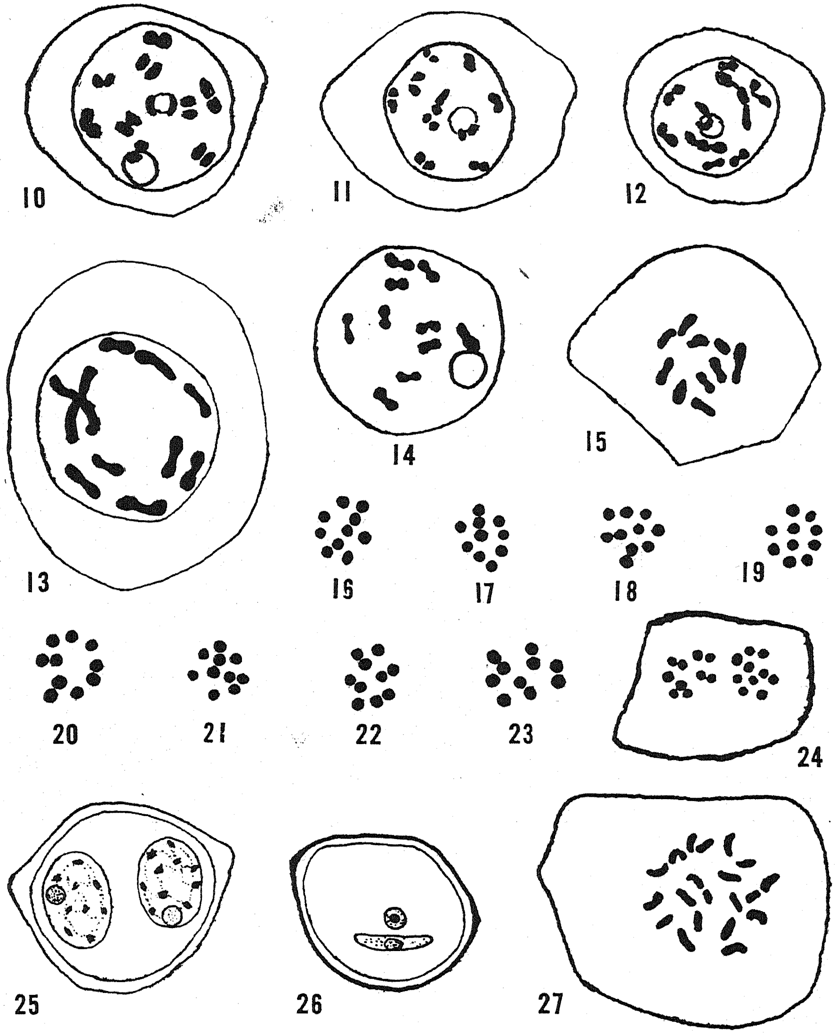
Table 1

No. of assns.	No. of bivalents in assn.			No. of cases	Total
	1	2	3		
1	8	1	—	4	4
2	6	2	—	11	
	7	—	1	1	12
3	4	3	—	7	
	5	1	1	1	8
4	3	2	1	3	
	4	—	2	1	
	2	4	—	1	5
5	1	3	1	2	2
					Total 31

Secondary association is maintained in anaphase I and metaphase II (Fig. 24). A similar persistence of secondary pairing during anaphase I has been recorded by Raghavan (1938) in *Gynandropsis pentaphylla*. Catcheside (1937), however, reports that in *Brassica*, secondary pairing completely disappears during anaphase I. The segregation of chromosomes is quite regular at anaphase. Kuwada (1929) assumes that the force that is responsible for the anaphasic separation is the polar attraction, while Darlington (1932) insists on a polar repulsion, which is essential for metaphasic equillibrium. Alam (1936) believes that anaphasic separation is brought about by two forces, repulsion between attachment constrictions and attraction of the poles.

The two groups of chromosomes as soon as they reach the poles, organise themselves into interkinesis nuclei. The nucleolus appears, and the chromosomes, which are now more or less uniformly spaced, are connected by thin strands (Fig. 25). This uniform spacing of the chromosomes at interkinesis, was first recorded by Gates (1909) in *Oenothera* and again by Raghavan (1938) in *Gynandropsis*. The two daughter nuclei are not separated by a cross-wall. They divide more or less simultaneously (Fig. 24). Figure 24 shows two groups of ten univalents each in second metaphase polar view. Secondary association persists up to this stage. The further meiotic process is regular resulting in tetrads, which may be either tetrahedral or iso-bilateral. The mature pollen

grain at the time of shedding is bi-nucleate (Fig. 26). Somatic cells of the root-tip showed the diploid number to be twenty (Fig. 27).



Figs. 10–27. *Angelonia grandiflora*. 10. Early diakinesis showing one ring bivalent and 9 rods. ×2225. 11. Mid-diakinesis. ×2225. 12. Diakinesis showing 2 (iii) and 7 (ii). ×2225. 13. Diakinesis showing a 1 (iv) and 8 (ii). ×2225. 14. Late diakinesis. ×2225. 15. Prometaphase. Note the close aggregation of the bivalents. ×2225. 16–23. Metaphase I showing the varying degrees of secondary associations. ×2225. Fig. 16 shows the stable configuration, with two bivalents in the centre, the remaining eight arranged in a ring around it. Fig. 23 shows the maximum association 1(3), 3(2), 1(1). ×2225. 24. Metaphase II polar view ×1515. Note the persistence of secondary grouping. 25. Interkinesis. Note the equal spacing of the chromosomes. ×1515. 26. Bi-nucleate microspore at the time of shedding. ×1515. 27. Metaphase polar view. Root-tip of *Angelonia grandiflora*. (2n = 20). ×1515.

(2) *Stemodia viscosa* Roxb.

The development of the anther is similar to that of *Angelonia*. The primary archesporium consists of a band of about three cells (Fig. 7), which divide periclinally giving rise to an outer layer of wall-cells and an inner layer of sporogenous cells (Fig. 8). The wall-cells by periclinal divisions give rise to three layers of cells of which the innermost becomes the tapetum (Fig. 9). The tapetum becomes bi-nucleate, when the pollen mother-cells are in late prophase (Fig. 9). The mature pollen grains at the time of shedding are bi-nucleate (Fig. 6).

The haploid chromosome number is twenty-one (Fig. 34).

(3) *Angelonia cubensis* (var. *alba*)

Materials of this were got from the Madras Agri-Horticultural Society. The plants are smaller in size than *Angelonia grandiflora*. The flowers are white instead of violet. The haploid chromosome number was found to be ten (Fig. 32). The metaphase I plates in this species also revealed secondary association of bivalents. Fig. 32 shows a plate showing 3(2), 1(3) and 1(1). The single unassociated bivalent is much bigger than the rest. Somatic chromosomes of this species could not be examined to find out if there was a definitely larger pair of chromosomes in the roottip complement. In *Angelonia grandiflora*, however, no such longer pair is present (cf. Fig. 27). For that reason, in the metaphase I plates, all the bivalents are of equal size. It seems therefore, likely, that there should be a difference in the chromosome morphology between the two species. Two other bivalents slightly smaller than this but bigger than the rest are associated, and placed near the biggest bivalent. This means that in the diploid complement there should be a pair of long chromosomes, besides two pairs of slightly less long chromosomes, so that altogether, in a somatic complement of twenty, six chromosomes are definitely longer than the rest. It is also of interest that in about half a dozen plates examined, these three bivalents, though not forming a single association, are situated close together, the biggest unassociated, and the other two forming an association of two; This probably indicates a kind of homology between the long chromosomes.

(4) *Dopatrium lobelioides* Benth.

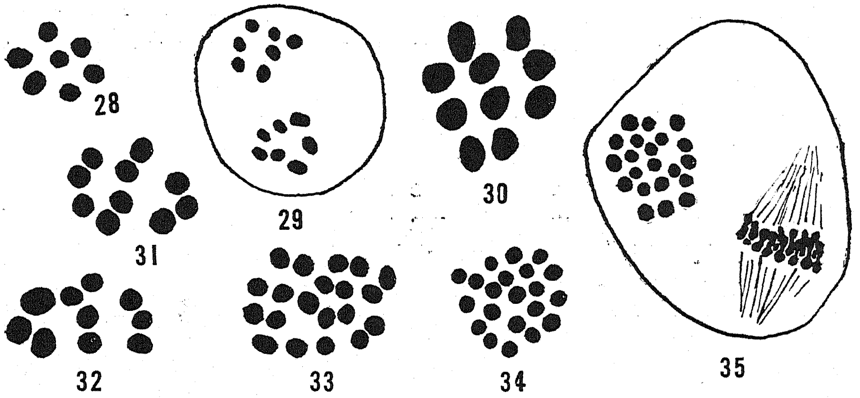
The haploid chromosome number was found to be seven. Fig. 28 and 29 show the first and second metaphase respectively.

(5) *Russelia juncea* Zucc.

Fig. 31 shows ten bivalents in first metaphase.

(6) *Russelia rotundifolia* Cav.

This species of *Russelia* also has ten as the haploid chromosome number (Fig. 30).



Figs. 28-35. 28. Metaphase I. *Dopatrium lobelioides* (n = 7) ×2225. 29. Metaphase II. *Dopatrium lobelioides* (n = 7) ×2225. 30. Metaphase I. *Russelia rotundifolia* (n = 10) ×3935. 31. Metaphase II. *Russelia juncea* (n = 10) ×3000. 32. Metaphase I. *Angelonia cubensis* (n = 10) ×3000. Note secondary association 1(3), 3(2), 1(1). Also the biggest bivalent unassociated. 33. Metaphase I. *Scoparia dulcis* (n = 20) ×3000. Note grouping of bivalents in twos. 34. Metaphase I. *Stemodia viscosa* (n = 21) ×3000. 35. Metaphase *Vandellia crustacea*, one of the groups in polar view (n = 21) ×3000.

(7) *Scoparia dulcis* Linn.

In the first metaphase twenty bivalents are seen (Fig. 33). Here also there is evidence of secondary association, the bivalents often forming groups of twos.

(8) *Vandellia crustacea* Benth.

Twenty-one univalents are seen in metaphase II (Fig. 35).

IV. Discussion

(a) Secondary association

The secondary association of bivalents was first observed by Kuwada (1910) in *Oryza sativa*. After that a number of workers have recorded this secondary association in metaphase I, anaphase I, and metaphase II. Darlington (1928) for the first time suggested

the theory of secondary association, which was later discussed in detail by Lawrence (1931).

Secondary association may be defined as pairing of ancestrally related bivalents. It results from an attraction between them. Secondary association is, therefore, an expression of ancestral homology and is therefore of great value in determining the constitution of polyploids, when it is combined with other evidence. In typical allopolyploids, multivalent formation is comparatively low. This is because, the homologous chromosomes derived from the two parents are not completely identical, but are slightly different. This is exhibited by the degree of attraction existing between the bivalents of the two parents. Usually, therefore, pairing takes place between chromosomes of the same parent. This is possible only when doubling of the chromosome set has already been brought about. Cases are, however, known which show a gradation from almost complete identity of the pairing chromosomes, derived from the parents, to strong difference between them.

In the diploid hybrid of *Crepis rubra* × *Crepis foetida* (Poole, 1931), complete pairing of chromosomes is present, suggesting that they behaved as though they were from the same parent. The tetraploid derived from it behaved almost like an autotetraploid, quadrivalents being very common. While in the diploid hybrid *Raphanus sativus* × *Brassica oleracea* (Karpechenko, 1927), no pairing occurs and in the tetraploid quadrivalents are absent. The occurrence of secondary association in such a tetraploid helps in the determination of the chromosome homology. The homologues of the two parents are different enough not to allow pairing between them but their affinity, though not sufficient enough for pairing by chiasmata, is enough to bring about the juxtaposition of the homologues at metaphase I, which phenomenon is secondary association. In other words, secondary association helps in the determination of the degree of affinity between the homologous chromosomes derived from the two parents. Darlington and Moffet (1930) and Moffet (1931) have with the aid of secondary association shown that in the Pomoideae the haploid chromosome number of 17 is a secondary basic number derived from an ancestral form with seven haploid chromosomes. Lawrence (1931) has shown that in *Dahlia merckii*, the evidence from secondary association substantiates the assumed basic number of eight for the genus *Dahlia*. He has also shown that in *Dahlia coccinea* secondary association gives the necessary evidence to infer that duplication of chromosome sets has taken place.

In *Angelonia* we find that the maximum number of associations

seen twice gives one group of three bivalents, three groups of two bivalents each, and a single bivalent. The diploid set of twenty chromosomes, therefore, belong to three types, one of which is represented six times, three four times and one twice. The haploid chromosome set can be represented thus:

A	B	C	D	E
A	B	C	D	
A				

The basic chromosome number, on the basis of maximum association for the genus *Angelonia* can thus be regarded as five. In the absence of fuller knowledge of the genetics and cytology of the other species of this genus, the evidence to support the view that *Angelonia grandiflora* is a polyploid with a basic number lower than ten, can only be inferred from (I) secondary association, (2) occasional formation of multivalents, chiefly trivalents and quadrivalents. In addition to these, the organisation of the nucleolus by the satellited chromosomes at mitotic telophase gives a clue to the polyploid nature of a plant. This has been fully discussed by Gates (1937). Also the presence of multiple factors in the genetical behaviour indicates unmistakably the polyploid nature. In the present study, evidence from the last two is lacking and as such, conclusions have to be drawn chiefly on the basis of the first two types of evidence. Secondary association has been utilised for the discovery of the polyploid nature of many plants by a number of authors. For instance, some of the apparent diploids are in reality polyploids and in some cases have undergone structural changes. They are to be regarded as secondarily balanced polyploids. In the cultivated rice (Nandi, 1936) $2n = 24$ is derived from a basic number 5; In *Brassica* $2n=18$ from a basic number 6. Sometimes, reduplication of single chromosomes have taken place, resulting in fertile species. These are to be regarded as secondarily balanced diploids; for example, *Tricyrtis* $n = 13$ and *Dicentra* $n = 8$ (Matsuura, 1935) are derived from basic chromosome sets of 12 and 7 respectively. Similarly, Meurman (1933) found that in *Acer* $n = 13$ is derived really from a true basic number 12. In the present study, the maximum association is 1(3), 3(2), and 1(1). The most frequent association being 6(1), 2(2). No typical case of five groups of two have been met with. *Angelonia* with $2n = 20$ is to be regarded as a secondary tetraploid having arisen by allopolyploidy. For successful and fertile allopolyploidy, syndiploidy in many cases, would appear to be a necessary antecedent. We can regard that the original parent A had a haploid set of 5 chromosomes. Another B could have arisen also having 5 chromosomes but having suffered structural

changes or gene mutation. A cross between the two, under ordinary circumstances, could only be expected to be partially sterile, with a very low frequency of pairing. If in the cross $A \times B$ doubling of the chromosomes takes place, it would be fertile due to autosyndesis. This doubling, however, might take place, either somatically, as in *Primula kewensis* (Newton and Pellew, 1929) and in *Nicotiana digluta* (Clausen, 1928) with its parents *glutinosa* and *tabaccum*, or it might take place by the fusion of polyploid gametes (Karpechenko, 1927; *Raphanus* × *Brassica*).

Thus, a fertile species of *Angelonia* with $2n = 20$ could have arisen.

In a typical secondary allotetraploid of this kind, the parental gametic genomes can be represented by A_1, B_1, C_1, D_1, E_1, and A_2, B_2, C_2, D_2, E_2. A cross between these, followed by syndiploidy, would result in the formation of a fertile true breeding hybrid, having the constitution $A_1A_1\ A_2A_2$, $B_1B_1\ B_2B_2$, $C_1C_1\ C_2C_2$, $D_1D_1\ D_2D_2$, $E_1E_1\ E_2E_2$. On account of the homology between A_1 and A_2 and B_1 and B_2 etc. typically five groups of two bivalents must be formed; bivalent A_1A_1 secondarily associated with bivalent A_2A_2, bivalent B_1B_1 associated with B_2B_2 and so on. Here, however, no such typical case has been observed. The maximum association is 1(3), 3(2) and 1(1); besides, in diakinesis, there are not infrequently formed some multivalents, chiefly trivalents. These observations indicate that structural changes have played a part in the evolution of the species in addition to polyploidy. Assuming structural changes in the nature of reciprocal translocations have taken place between D_2 and E_2 chromosomes, then the diploid number 20 may be represented as $A_1A_1\ A_2A_2$, $B_1B_1\ B_2B_2$, $C_1C_1\ C_2C_2$, $D_1D_1\ D_2(D_2E_2)$, $E_1E_1\ E_2(E_2D_2)$. Because of the segmental interchange there is brought about, possibly, an affinity between the D_2 and E_2 chromosomes. Thus the maximum association of 1(3), 3(2), 1(1), may be represented as $A_1A_1\ A_2A_2$, $B_1B_1\ B_2B_2$, $C_1C_1\ C_2C_2$ (three twos), D_1D_1, $D_2(D_2E_2)$, $E_2(E_2D_2)$, (one three), and E_1E_1 (one one). The occasional quadrivalents met with must be due to the association of the D chromosomes with the E chromosomes; a condition very near the typical condition of 5(2) is occasionally to be seen in an association of 4(2) and 2(1) (Fig. 22). Presumably, owing to some causes, attraction between the two related bivalents like E_1E_1 and E_2E_2 has failed. This also may be attributed to the structural changes referred to above.

The evidence from secondary pairing alone, as a criterion of ancestral homology cannot, however, be regarded as complete. In the first place, structural changes of the homologous chromosomes

for a longer period of evolution may have taken place to a great extent in some polyploids and as a result, the degree of affinity required to cause attraction may not be present. Secondly, structural changes like simple translocations and reciprocal translocations, which are prominent factors in the evolution of new species may give rise to higher associations, so that the basic number inferred from the observed secondary association may be erroneous and lastly there is a possibility that secondary association may also be, like primary pairing, gene controlled.

A hypothesis recently put forward by Heilborn (1936) may also be briefly mentioned in this connection. Heilborn, who has studied secondary association in *Carex,* came to the conclusion that chromosomes of equal size are associated, irrespective of their homology and that it is not a specific attraction or pairing between homologous parts of chromosomes or homologous genes, but the parallellism of the associated chromosomes is mechanically induced through the polarity of the nuclei. Flowik (1938) commenting on this, says that on the other hand, it is just in *Carex,* that one should expect secondary association in the true meaning of the word, because in *Carex*, the chromosome numbers are throughout high, and for that reason one may be justified in suspecting the presence of polyploidy in some form or other. The chromosomes in *Carex* are very small and as a consequence, the chiasma frequency must be assumed to be low. It follows, therefore, that polyploidy can hardly make itself evident in prophase pairing as multivalents, but on the other hand, the polyploid nature of *Carex* can be revealed by the occurrence of secondary association. Müntzing (1936) considers that there is evidence for considering the basic number of this species as secondarily balanced. This evidence is to be seen in the behaviour of A′ and A″ groups of chromosomes, to which Heilborn attaches great importance, in support of his hypothesis. Müntzing (1936) in this connection says "If the species is a simple autotetraploid, the basic number should be 18. Considering that this value is rather high, it is probable that *Carex glauca*, though in the main an autotetraploid species may have a more complicated constitution and originally a lower basic number". Thus Heilborn's hypothesis as such cannot to accepted as a weighty argument against Darlington's (1932) original interpretation of the phenomenon of secondary association.

It is a known fact that the secondary association of chromosomes, manifests itself first at prometaphase of the first division and is maintained in the succeeding metaphase I. But at the

preceding diakinesis the bivalents show no secondary association, though Catcheside (1937) has found that the beginnings of secondary association are to be seen even as early as diakinesis. Darlington (1932) is of the opinion that the mechanism of distribution of chromosomes on the metaphase plate is modified by the attraction, which is specific for chromosomes. Matsuura (1935) argues that it is difficult to explain why the same force of attraction between related chromosomes, which brings about secondary association in metaphase I, does not exert its influence to bring about the association of the related bivalents during diakinesis. He considers that there is no essential difference between the diakinetic stage and metaphase I with regard to their repulsion, except for the fact that the former represents a repulsion phase in three dimensions, while the latter, one of two dimensions and it is also known that there is no material connections between the associated chromosomes.

Matsuura explains this difficulty by putting forth the view that the karyomere takes an active part in causing the association of the related bivalents. He found eight bivalents in *Dicentra spectabilis*. In a few metaphase plates he found no secondary association. In those which showed secondary pairing, he invariably found two particular chromosomes alone associated together. Further, he observed that the chromosomes did not come directly into touch with the plasm, but he found them lying in the same "karyomere cavity or chromosome sheath". In some favourable cases he clearly found the karyomere surrounding each bivalent and also found the paired bivalents lying in the same karyomere cavity. Matsuura, therefore, suggests that during prometaphase, when the bivalents are closely assembled together in the centre, the force of attraction existing between the related bivalents brings about the fusion of their karyomere cavities and on account of the retention of the fused cavity, the bivalents remain associated in the succeeding metaphase stage. He explains the absence of any association at all in certain plates by assuming that the mechanism of karyomere fusion has failed in those cases. Matsuura also finds support for his conclusions from figures given by previous workers.

Haga (1938) formulated the genome-constitution of different species of *Brassica*, exhibiting marked aneuploidy with the help of secondary association.

(b) The basic chromosome number of the family

The basic number of a family can be inferred from the following:— a comparison of the size and shape of the meiotic and

mitotic chromosomes, a study of their behaviour during meiosis especially the primary and secondary associations. Wanscher (1934) has made a statistical study of the chromosome numbers of forty four angiospermous families and came to the conclusion that the chromosome numbers of the higher plants originate from numbers belonging to a four-system. From this four-system, they develop in various ways. Usually, the most probable course is by loss and gain of chromosomes to form descending and ascending series and from these secondary numbers, multiple series may be formed as from the four-system itself. He considers that in the greater number of families, the cytological and other data point more or less distinctly to basic numbers, belonging to, or closely related to the four series. Babcock (1934) criticising Wanscher's deduction of basic chromosome numbers, says that a mere statistical analysis of the distribution of chromosome numbers is by itself inadequate for the determination of basic chromosome numbers, and cites *Crepis* as an example, in which though less than one-fifth of the species in the genus possess the haploid chromosome number of five, five must be considered to be the basic number of the genus for more important reasons.

In the Scrophulariaceae, different genera show different basic numbers; *Linaria* 6, *Gratiola* and *Dopatrium* 7, *Antirrhinum* and *Pentstemon* 8, *Odnites* 10, *Euphrasia* 11, *Digitalis* 12. The other genera show more variations in their numbers. For instance, the genus *Verbascum* shows 15, 16, 18, 24, and 32. *Lathrea* 8 and 21. The genus *Veronica* has 7, 8, 9, 12, 16, 17, 20 and 24. According to Wanscher the chromosome numbers in *Veronica* conform to the descending series 8–7. As the basic numbers of a number of genera is found to be eight, he concluded that eight is the primary basic number. In the present investigation, the basic number of the genus *Angelonia,* which belongs to the *Antirrhineae,* is found to be five. The basic number of the genus *Antirrhinum* itself is eight. No genus so far investigated in the Scrophulariaceae shows a basic number of five. Two alternatives must be considered in this connection. Firstly, that five should be the primary basic number of the family, in which case all the other numbers represented by the different genera must be considered as secondary balances of this primary number. From these secondary numbers, multiple series may be formed as from the five itself. The other alternative is to consider the possibility of adhering to the number four as the primary basic number. This should be in accordance with the general trend of the distribution of chromosome numbers in the family and which made Lawrence (1930) regard eight as the pro-

bable basic number of the genus *Verbascum* as also of many other members of this family. This would involve the consideration of the genus *Angelonia* as a member outside the family. For this, however, there is not much evidence, though there are some tempting details in favour of its exclusion They are the absence of a distinct tapetal tissue surrounding the embryo sac and the absence of any kind of endosperm haustorium, features, which are characteristic of members of this family. But then, the absence of a tapetal layer and endosperm haustoria cannot be regarded as enough evidence to exclude *Angelonia* from this family. For absence of a tapetal layer has been reported in *Striga lutea* (Mitchell, 1915) and absence of endosperm haustorium in *Scoparia* (Schertz, 1919). Apart from these, the morphological features of *Angelonia* are so arrestingly characteristic, that they would not permit of its exclusion from the Scrophulariaceae. The typical bi-locular ovary, with an indefinite number of ovules arranged on an axile placenta, and the extreme zygomorphy of the corolla all decisively point to the fact that *Angelonia* is best retained in the Scrophulariaceae. In that case five should be regarded as the primary basic number of the family, and all the other numbers as secondary balances of this primary number.

V. Summary

1. The chromosome numbers of the following genera have been determined for the first time:—

		n	2n
(1)	*Angelonia grandiflora* C. Morr.	10	20
(2)	*Angelonia cubensis* var. *alba*	10	—
(3)	*Russelia juncea* Zucc.	10	—
(4)	*Russelia rotundifolia* Cav.	10	—
(5)	*Stemodia viscosa* Roxb.	21	—
(6)	*Dopatrium lobelioides* Benth.	7	—
(7)	*Scoparia dulcis* Linn.	20	—
(8)	*Vandellia crustacea* Benth.	21	—

2. Details of meiosis have been worked in *Angelonia grandiflora*. Secondary association has been recorded.

3. The scope and limitations of secondary association as indicating the basic number of a genus or of a family are discussed in the light of the observations made in *Angelonia*.

4. The question of the basic number of the family as revealed by the investigations of previous workers is discussed in the light of the phenomenon of secondary pairing observed in the Scrophulariaceae for the first time in the present investigations, and the

conclusion is reached that five is likely to be the primary basic number, and all other numbers represent different balances of this primary number.

5. The origin of the anther sac and the tapetum is described. It becomes bi-nucleate early and remains so until its final disintegration.

6. The pollen grains of *Angelonia* and *Stemodia* are bi-nucleate at the time of shedding.

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Studies in the Indian Scilleae
III. The Cytology of diploid *Urginea indica* Kunth.

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I. Introduction

The diploid chromosome number of *Urginea indica* was recorded a few years ago for the first time by one of us (Raghavan 1935). The analysis of the somatic complement was made therein and the possible occurrence of triploid individuals was also indicated. The study of meiosis had to be put off for some reasons. Last hot weather an attempt was made to study the meiotic phenomena. In the course of our investigation, triploid individuals were encountered as anticipated and a study of the meiosis and other relevant features of the triploid are reported in another paper. In the present communication we have concerned ourselves with the diploid. While seeking to confirm cytologically our macroscopic isolation of the diploids from a heterogenous population, we came across certain interesting details, an account of which is also given. They relate to the occurrence of plants with variable number of chromosomes. In some, we find varying chromosome numbers in the same individual, while in others it is suspected that entire individuals show numbers other than the true diploid number, namely 20. In others we find entire roots exhibiting the tetraploid number. While, therefore, true diploids occur, there are present amongst them such cytologically irregular plants. These are being isolated for a further

detailed correlated study. For the present, an account of meiosis in the diploid is given with a description of the fragments and their behaviour.

Root-tips were fixed in Navashin with pre-fixation in Carnoy and stained in Newton's Iodine-Gentian Violet. Anthers of the desired stage of development, which was arrived at by acetocarmine examination, were also treated likewise. For smears, the fixative that was most successful was Belling's Navashin.

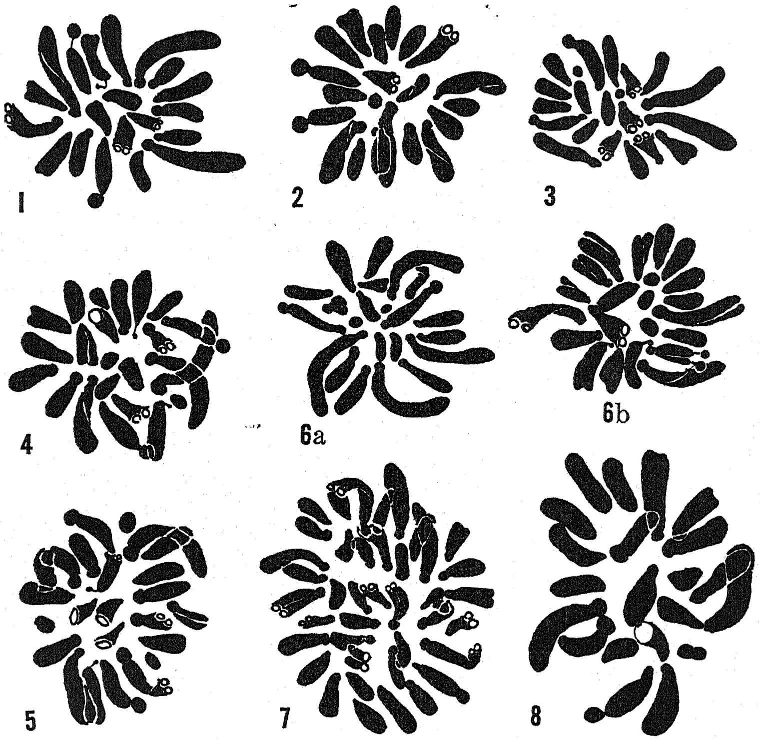
II. Somatic Chromosome Number

The somatic complement has already been analysed as being composed of ten pairs of chromosomes in the diploid (Fig. 1) and ten threes in the triploid. Almost all the chromosomes are characterised by subterminal constriction, and the range of size is appreciable. Of the C chromosomes (Raghavan 1935) two chromosomes can be distinguished from the rest, C_1 having a secondary constriction at its distal end, and C_2 characterised by a terminal constriction and a satellite at its proximal end. How far the secondary constriction of C_1 can be called a satellite, is a matter of opinion.

While examining a number of these diploid roots, we came across a few interesting phenomena. While 20 is the usual diploid number, we found a number of plates showing 21, 22, 23 and 24 complements. In sections exhibiting 21 and 22 numbers, it was not usual to find the 20 complement. This might at first sight indicate the prevalence in the population, of plants with diploid number of 21 and 22. This is in no way uncommon especially in a species like this where both diploids and triploids grow side by side. These plants may be regarded as the progeny of the triploid. This was however dispelled by the occurrence of 20 roots alongside of 21 and 22 roots in the same bulb. While therefore the possibility of the existence of 21 and 22 chromosomed plants is not ruled out, the present cases must be regarded as arising out of some mitotic aberrations, chiefly fragmentation.

The occurrence of chromosome variation may be analysed into, 1. entire individuals exhibiting a particular number and 2. varying numbers being found in the same individual. Undoubtedly the former have arisen by the union of gametes containing different numbers. How these numbers might have arisen is discussed elsewhere. The second type is presumably due to fragmentation of chromosomes. In this most commonly the C_1 and C_2 chromosomes do not appear to take part as an idiogram study of these complements

would indicate. Though a preliminary examination has indicated the existence of entire individuals showing these varied numbers, isolation of these individuals has not yet been effected, as amongst the diploid population fixation was not effected plant by plant. Now that the existence of individuals showing these different numbers has been suspected, a cytological examination of all these individuals one by one is in progress. For the present, observations are confined to the second type and an attempt has been made to find out by idiogram study which of the chromosomes have undergone fragmentation.



Figs. 1–8. Somatic metaphase plates. 1. 2n 20; the C_1 and C_2 chromosomes can be recognised. 2. 2n 21; with 1 fragment. 3. 2n 21; with 2 fragments. In both the C_1 chromosomes are in tact. 4. 2n 23. 5. 2n 24. 6a. 2n 42; note the pair of C_1 chromosomes is in tact. 6b. 2n 22; only one of the C_1 chromosomes is present. 7. 2n 42. Figs. 1–7. ×1650. 8. 2n 21; note that one of the fragments showing median constriction. ×2200.

Normally the diploid complement consists of two long chromosomes, A, four slightly less long, B, ten medium chromosomes, C, and four short chromosomes, D. Of the C chromosomes C_1 has a secondary constriction at its distal end and C_2, a satellite at its proximal end. There is a gradual reduction in size from A to D

and the two pairs of B chromosomes are not exactly similar, in size. So also the five pairs of C chromosomes, so that there is no clear demarcating line between C and D, the difference between the shortest C chromosome and the D chromosome being very little (Figs. 1 and 9).

In the 21 complement, there are two types, in one (Fig. 2) there is only a single fragment, in the other (Fig. 3) there are two fragments. The idiogram of Fig. 2 is represented diagrammatically in Fig. 10. It can be gathered from a close study of the figures that one of the B chromosomes is missing. It is inferred that it has undergone fragmentation and having become shorter has merged with the medium C chromosomes. In the other with two fragments, the entire six A and B chromosomes are present and it is likely that one of the D chromosomes or the shortest C chromosome has fragmented into two.

In the 22 complement we get generally two fragments (Fig. 6a and 6b). The method of origin of these fragments seems, however, to be different. In Figs. 6 and 11 there are again five long chromosomes whereas there should be six (A & B). This means that one

Figs. 9–13. Idiograms of some of the somatic complements represented in Figs. 1–8; for explanation see text.

of them has suffered fragmentation. The other fragment is accounted for by one of the C_1 chromosomes breaking away the secondary constriction. That is why in that complement the C_1 chromosome has no homologue (Figs. 6b and 11). The other method by which the second fragment would appear to be formed can be inferred from Fig. 8 where both the C_1 chromosomes are present and where

also could be seen a short chromosome-like body with a deep median constriction. In the somatic complement of *Urginea indica* medianly constricted chromosomes are conspicuous by their absence, all the chromosomes possessing constriction of the subterminal type. It is therefore possible that the fragment which was derived from one of the B chromosomes is undergoing further fragmentation into two. That is why in such a complement (Fig. 6a) both the C_1 chromosomes are in tact.

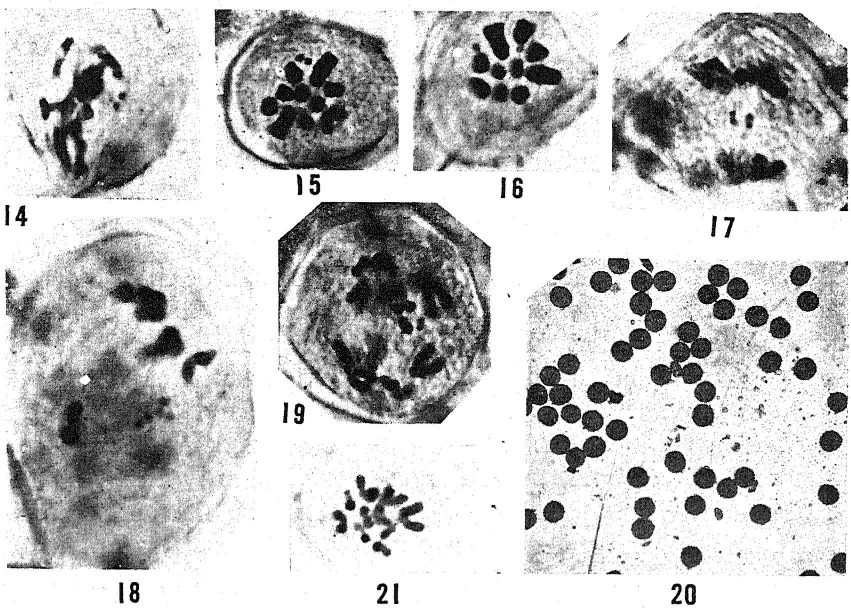
In the 23 complement (Figs. 4 and 12), three long chromosomes are present instead of the six (2 A plus 4 B), and there are three fragments. It is likely that these were given off by the three B chromosomes.

In the 24 type (Figs. 5 and 13), there are three long chromosomes also (1A and 2B) and there are four fragments. This is the first time that one of the A chromosomes has suffered fragmentation, there being no homologue for A. So three fragments arise from 1 A and 2 B. The fourth is accounted for by one of the D chromosomes undergoing fragmentation. One interesting thing that stands out prominently is that whereas one would expect chromosomes with secondary constriction like the C_1 and those with satellites, C_2 to take a prominent part in fragmentation, it is strange that they remain almost unaffected. In almost all the complements examined, except in Fig. 6 (2n=22), we find these conspicuous by their presence. And there would appear to be no restriction to the chromosomes that do fragment. Sometimes it is the A chromosome as in 2n =24, or the B chromosome as in 2n = 21, 22 and 23 and less frequently it is the C or the D chromosome as in 2n = 24.

III. The Behaviour of Fragments at Meiosis

As explained already fragments came to be noticed only during the cytological examination of the diploid population and since fixation of pollen mother cells was effected without reference to the somatic chromosomes of the particular individual, it is not possible for the present to describe the behaviour of the fragment chromosomes in the different individuals separately, as the 21, 22 or the 23 individual. For the time being the behaviour of fragments in general will be described. Figs. 24, 25 and 26 show pollen mother cells in First Metaphase showing one, two and three fragments respectively. Fig. 15 is a photomicrograph of MI with two fragments. In Fig. 22 we see fragments even at diplotene stage. Fig. 14 is a photomicrograph of the same. In early diakinesis (Fig. 23), we find the two fragments synapsing so that we altogether

see eleven gemini. Fig. 27 is side view MI with 3 fragments lying outside the spindle. In anaphase their behaviour is varied. In Fig. 32, 2 fragments are included in one pole while another lies altogether outsides the poles. Sometimes there is normal disjunction half the number of fragments going to each pole, or these may be unevenly distributed. In Fig. 29 one fragment is included in one pole while three are in the equator. All the fragments may go to

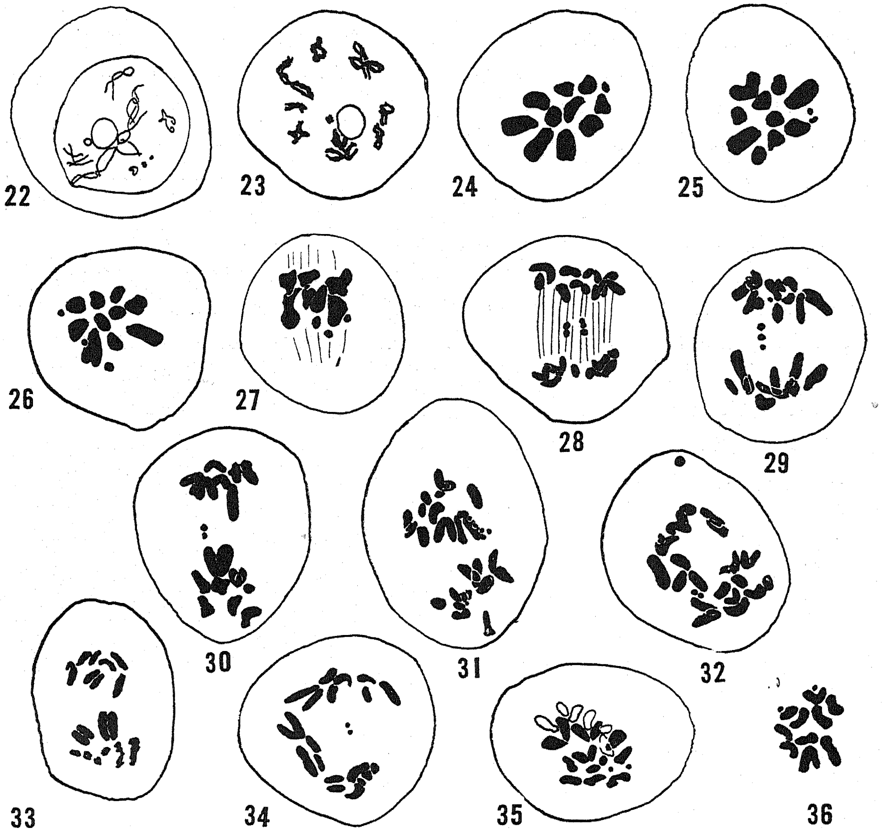


Figs. 14–21 (photomicrographs). 14. p.m.c in late pachytene showing two fragments. 15. p.m.c MI showing two fragments. 16. p.m.c MI; the C_1 and C_2 bivalents with their secondary constriction and satellites respectively. 17. AI; two fragments dividing at the equator. 18. AI showing 5 fragments all going to one pole; only the fragments are in focus. 19. A fragment dividing by constriction. Another has divided and the two divided halves have reached the top pole together. 20. Pollen grains showing the generative and vegetative cells. 21. Anaphase, polar view showing 14 chromosomes; same as Fig. 35.

one pole (Figs. 31 and 32). Fig. 36 is that of AI with ten plus 2 fragments. Fig. 31 is side view of AI showing all the five fragments in one pole, while in the other pole there is no trace of fragments at all. Fig. 18 is a photomicrograph of the same; only the fragments are in focus. Sometimes fragments divide by a sort of constriction and the halves separate in the usual manner. Figs. 17, 28, 30 and 34 represent fragments dividing prior to disjunction.

There is also evidence of non-disjunction at least in respect of some bivalents. In Fig. 33 we find homologous chromosomes in a

paired condition and reaching the same pole. Fig. 35 is AI polar view with 14 chromosomes in one pole and six in the other. This is a clear case of non-disjunction of four bivalents. The occurrence of homologous chromosomes in the 14-chromosomed pole is clearly visible, as also the presence of his fragments therein. Fig. 21 is a photomicrograph of the same. If this takes place in a haphazard manner naturally we get irregular chromosome distribution leading



Figs. 22–36. ×990. 22. Early diplotene with two fragments. 23. Early diakinesis with 11 gemini presumably as a result of the synapsis of the fragments. 24–26. MI with 1, 2 and 3 fragments respectively. 27. MI side view with three fragments off the plate. 28. AI with 5 fragments, 2 fragments dividing and 1 undivided. 29. AI, one fragment having reached the upper pole, 3 remaining at the equator. 30. A single fragment dividing at the equator. 31. 'All the 5 fragments derived as in Fig. 28 reaching one pole. 32. The two halves of a divided fragment reaching a pole, another fragment lying outside the nuclear spindle. 33. Paired chromosomes reaching the poles together, a case of non-disjunction. 34. Single fragment dividing prior to disjunction; note also the asymmetric disjunction. 35. First anaphase, polar view. At the top pole are seen 14 chromosomes with two fragments while in the lower pole 6 chromosomes are seen. 36. Anaphase one, polar view showing 10 chromosomes and 2 fragments.

to the formation of individuals with different numbers of chromosomes.

The other phenomenon worthy of note is the occurrence of tetraploid roots, in diploid plants. Tetraploid cells are quite common in diploid roots; they occur scattered and there would seem to be no particular sector or portion of the root to which tetraploidy is confined. Presumably these arise from some mitotic aberration, like non-disjunction. While such stray plates are common, we found that one or two entire roots of diploid bulbs were wholly tetraploid. Morphologically these tetraploid roots are not different from diploid roots of the same bulb. The possible significance of the occurrence of these is discussed in the other paper.

There were also some plates showing 42 chromosomes. These were not also stray plates and the same number characterised the whole root (Fig. 7). It is therefore suggested that some of the roots of an individual plant, exhibit this number. Presumably this is the result of the failure of anaphasic separation in a complement with 21 chromosomes. The occurrence of 21 and 22 chromosomed roots side by side with 20 chromosomed roots has already been mentioned. The occurrence of stray plates could be explained by the above said anaphasic non-separation in a 21 complement. But the prevalence of entire roots showing the same number throughout indicates that some of the roots of a diploid plant have come to assume this number in the same manner in which the 21 number arose, but only, in addition to the fragmentation there has taken place here a failure of anaphasic separation also.

IV. Meiosis in the Diploid

Earliest diplotene stages point to a complete pachytene pairing. The chiasmata are distributed evenly among the pairs of chromosomes, during this stage (Figs. 37 and 38). The various configurations seen at diakinesis and metaphase can be explained by this phenomenon of pairing of homologous chromosomes and the formation of chiasmata. Each chromosome is longitudinally split into two chromatids which associate in pairs and chiasmata are formed between the homologous chromosomes by an exchange of partners between these chromatids. The number and position of the chiasmata determine primarily the shape of each configuration, as also the position of the attachment constrictions which in this case, however, do not show any variation, as they are all of the subterminal type. It looks as though these chiasmata are formed more or less at random along the length of the pairing chromosomes at diplotene.

Forces of repulsion acting between the centromeres result in the chiasmata travelling to the distal ends of the chromosomes. This terminalization results in a reduction in the number on account of their being telescoped at the chromosome ends. The same result can be secured by merely pushing the distal interstitial chiasmata along to ends to form terminal chiasmata, while the interstitial loops adjust their relative sizes tending towards a state of equilibrium (cf Darlington and Dark 1932). Fig. 37 is that of a pollen mother cell at middle diplotene. The number of chiasmata is comparatively large. The total number of chiasmata is 35 of which four are terminal. The quadruple nature of the bivalents is seen at this stage. The number of chiasmata per bivalent varies from one to six. A characteristic feature of this stage is the comparatively large proportion of chiasmata per bivalent. The average number of chiasmata per bivalent is 3.5, while that of the terminal chiasmata is 0.4.

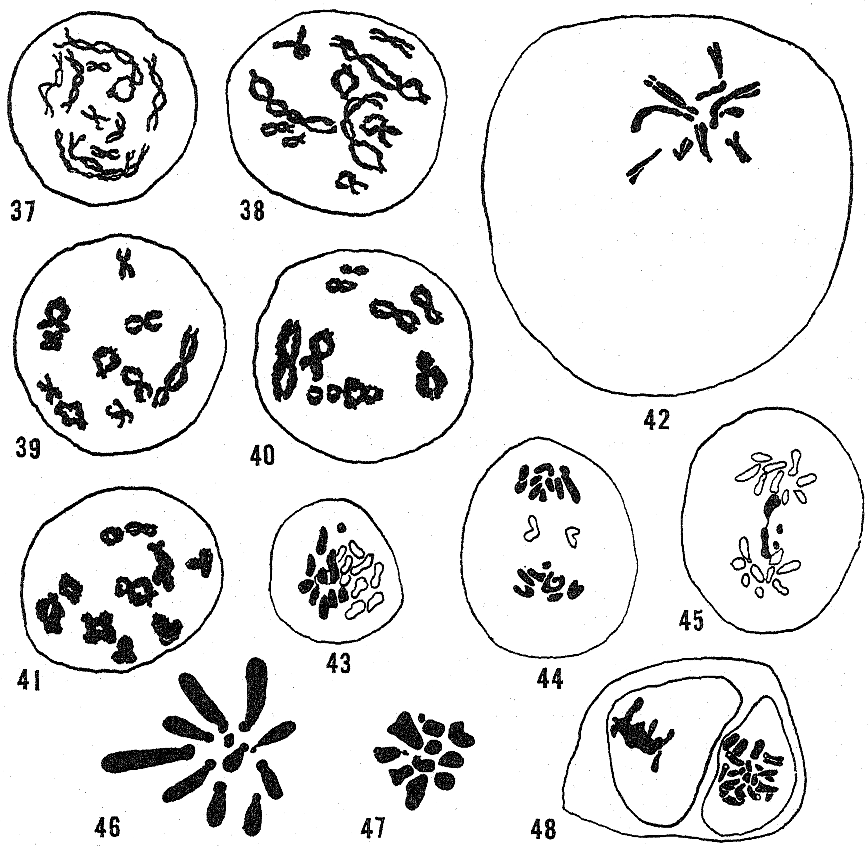
At late diplotene, contraction has proceeded to some extent and there is a reduction in the number of chiasmata (Fig. 38). The frequency is about 2.5 per bivalent. Coupled with this is an increase in the number of terminal chiasmata, its frequency being about 1.1 per bivalent. Though the total number of terminal chiasmata generally increases, in many instances, there is evident localization of the chiasmata towards the attachment constriction. In them terminal chiasmata are rather rare.

At diakinesis (Fig. 39–41) there is still further contraction and among other types the cruciform type is one of the commonest types of geminus. The number of chiasmata per bivalents has become still further reduced, with an increase in the number of terminal chiasmata.

At first metaphase the gemini arrange themselves as a flat plate in the centre (Figs. 16 and 47), and the ten bivalents could be easily counted. The chromosomes at this stage are too condensed to demonstrate chromatids and chiasmata directly. The noteworthy fact is that even the satellites are clearly visible at this stage. In the diploid complement, the occurrence of C_1, with a distal secondary constriction, and the C_2 with a proximal satellite has already been made mention of. Apparently it is these two pairs that appear as the satellited gemini in the first metaphase. This is supported by the fact that while the satellite of one geminus is from its proximal end and therefore directed towards the centre of the plate, in the other it is situated at the distal end and directed centrifugally. Also the size of the latter is somewhat bigger than the other which fact suggests that it is likely to be the C_1 geminus. The other interesting thing is the prominent development of the trabant which was not

quite so evident in the somatic chromosomes. It is not quite usual to find the satellites persisting in the first metaphase bivalents; and to find them in an exaggerated form especially with a prominent trabant which was not quite in evidence in the somatic chromosomes, means that there is something that is responsible for their persistence and development.

A situation somewhat analogous to this has been observed in *Phalaris canariensis* (Parthasarathy 1939). He records that while



Figs. 37–41. p.m.c at successively later stages showing progressive contraction with decrease in the total number of chiasmata and an increase in the number of terminal chiasmata. ×1980. 37. Late pachytene 35/4. 38. Late diplotene 25/11. 39. Somewhat later stage 18/10. 40, 41. Diakinesis; 41. 22/14. Fig. 42. Pollen grain; post meiotic mitosis with the haploid number of chromosome 10; the C_1 and C_2 chromosomes can be seen. ×990. Figs. 43–48. 43. First anaphase showing 10, 10 in each pole. ×990. 44. do with two lagging chromosomes. ×990. 45. do with 9 chromosomes in each pole and two chromosomes in the middle. There are also 2 rounded bodies lying along the spindle; explanation see text. ×990. 46. First anaphase showing 10 chromosomes and fragments. 3000. 47. First metaphase; same as Fig. 16. ×990. 48. Second metaphase, the lower pole showing 20 chromosomes owing to non-separation. ×990.

the satellites in the somatic chromosomes are very small, their size and proportions at diakinesis are considerably larger when compared with the rest of the chromosomes. While at diakinesis their prominence may be due to nucleolar organizing bodies (McClintock 1934), their exaggerated appearance in first metaphase is inexplicable. McClintock thinks that the nucleolar organizer is at the end of the chromosome proximal to the satellite, but it is possible that the stretched region may not be the satellite stalk, but may represent a region proximal to the end of the chromosome, while the attached region may include the end of the chromosome together with the satellite, the thread having been withdrawn.

Anaphase I is usually normal. Fig. 43 shows Anaphase I, polar view; the chromosomes in both the poles could be counted, ten each, and this testifies to the regularity of the anaphasic separation. Occasionally, however, one finds two chromosomes lagging at the equator (Fig. 44). In Fig. 45 we see two chromosome-like bodies connected by a thin thread in the middle stretching along the poles. In each pole could be counted nine chromosomes. Besides, in Fig. 45 we see two fragments lying along the spindle. Since we see nine chromosomes in each pole, the two bodies stretching along in one case, and lying separately in the equator in the other, must represent the two chromosomes of a bivalent. At first sight it looks like a case of chromatid bridge with fragments. In that case it would mean the presence of inverted segments in a pair of chromosomes, and the crossing over in these might be responsible for the bridge and fragment, or it may be a case of non-homologous association (McClintock 1933). But there is no evidence of structural changes as having taken place; nor is there anything to suggest that uneaqual chromatids are formed. It is very probable that the stretching along the spindle may be due to the two chromosomes composing the bivalent with the secondary constriction. In other words, the separation of the two C_1 chromosomes is delayed. It may be that the presence of the large secondary constriction at the distal end accounts for this delay in the separation of the constituent chromosomes. And it is these, presumably, that lie in the equator in Fig. 45. They will reach the respective poles, though late, and be included in the daughter nuclei. The prominent thread-like trabant is presumably responsible for the delay in the separation of these chromosomes. The reason why the other geminus with terminal satellite does not also behave likewise is not quite clear. Perhaps the smaller size of the satellite—being a true satellite and not a secondary constriction as in the case of the C_1 chromosome—does not hinder this anaphasic separation as much as in the other case.

The two small bodies seen in Fig. 45 are presumably the satellites—the secondary constriction portion of the separating chromosomes. They may or may not be included in the daughter nuclei. If the fragment also includes a small portion of the centromere then it can survive and function as a small chromosome, as it would then have all the necessary things for a functioning chromosome. The C_1 chromosome from which this has fragmented, would not also suffer as it has another centromere at its proximal end, to make it a complete chromosome. It is perhaps such an AI plate that is represented in Fig. 46 where we see the presence of a fragment in addition to the usual ten chromosomes. This is supported by the absence of the secondary constriction at the distal end of the C_1 chromosome. The C_2 chromosome is in its entirety having suffered no fragmentation.

This second division is normal and at second metaphase most often we get the 10–10 plates. Cross wall formation is completed at the end of the first division. The microspores that are ultimately formed are all of the same size, there being no polymorphism (Fig. 20). In Fig. 42 is shown a pollen grain in post-meiotic metaphase, showing the full haploid number of chromosomes. Occasionally there is failure of anaphasic separation in the second division, in one or both cells. In Fig. 48, we see in one cell 20 chromosomes. It is very unlikely that this will function as a diploid grain, as there is no evidence of the occurrence of polyploid gametes in the diploid plant.

V. Discussion

a) Somatic Chromosome Number

That a species is characterised by a single unchangeable chromosome number is not universally true. Clausen (1931) found such an irregularity in a species of *Viola*, *V. canina*, and has summarised the evidence in this respect. Since then a number of such species have been reported from time to time. We have ourselves reported the occurrence of an oscillating chromosome number in respect of another member of the Scilleae, *Scilla indica*, where we found $2n = 44$, 45 and 46. But there was some morphological difference between the 45 karyotype on the one hand, and the 44 and 46 on the other. The former had broader and longer leaves. 44 and 46 types were indistinguishable. We may perhaps regard the 45 type as a variety of the species. How far the chromosome number is constant within a variety cannot be said with certainty as we have not examined cytologically such large-leaved plants from different localities. There

are of course some species in which there is constancy of chromosome number within a variety and very often the numbers are in polyploid series. Presumably such forms have arisen through tetraploidy with subsequest interspecific hybridity. Difference in chromosome number may or may not be accompanied by a difference in external morphology. *Listera ovata* (Richardson 1933) is a species with an oscillating chromosome number. So also *Viola canina* (Clausen 1931) with 2n= 40 plus a variable number of fragments and chromosomes. So also in *Crepis syriaca* (Hollingshead and Babcock 1930), with 2n = 10, 11, 12, 13, 14, 15, 16 and 18. Clausen thinks that *Viola canina* is a hypertetraploid and maintains itself through intercrossing within the species. In *Crepis syriaca* the varying chromosome number is thought to be due to hybridization between two forms of a related species, *C. alpina*, followed by a chromosomal modification. Cameron (1934), and Sorokin (1927) found that in *Ranunculus acris* the variation in chromosome number resulted from crosses between different polyploid forms.

Sudden alterations of chromosome numbers are brought about by aberrations of mitosis, by the union of gametes with different chromosome numbers and by a combination of these two processes. In *Urginea indica* we have no evidence that we are dealing with a polyploid species as the few species of *Urginea* that are indigenous to India do not show number lower than the present recorded number. And in the localities where these collections were made—they extend over a wide area—no other species of *Urginea* is known. And the only other species that occurs at random, *U. polyphylla* has the same chromosome number. It is therefore improbable that recent interspecific hybridity has been responsible for the production of these forms. It may be that intraspecific hybridity between a form with 24 chromosomes and one with 20 has probably led to the establishment of the 22 forms. Similarly the 21 plant may have arisen from a cross between a 22 and a 20 plant.

b) Fragmentation

The occurrence of fragments in the somatic plates as also in meiosis, and their persistence leads us to suspect that the method of origin of these oscillating number is to be primarily traced to fragmentation. Fragmentation has been extensively studied among other genera in *Zea* (Randolph 1928), *Crepis* (Navashin 1925), *Tradescantia* and *Fritillaria* (Darlington 1929). Tuschnyokova (1929) showed in *Listera ovata* the addition of a small chromosome to the haploid complement of 17. From these 18-chromosomed

gametes the 36-somatic complement gains two small chromosomes. She postulated that the additional chromosome arose out of a non-disjunction of a small bivalent and through the fertilization of 18-chromosomed gametes individuals with 36 chromosomes arose. But Richardson (1933) working on the same species found that the ends were blunt, that the attachment constrictions were absent and therefore came to the conclusion that the additional chromosomes had arisen by fragmentation. Darlington (1929) found that the fragments arose during prophase of meiosis and that they were capable of forming new attachment constrictions. Their survival and subsequent behaviour was dependent upon their capacity for repeating mitosis and their ability to form chiasmata during meiosis.

In the present case, it is possible that entire individuals showing these numbers have arisen by the union of gametes showing different chromosome numbers. These in their turn owe their origin to some aberration of meiosis, non-disjunction or fragmentation. Of the occurrence of chromosomal fragments there exists plenty of evidence in the pollen mother cells. And there is also evidence of non-disjunction leading to the formation of gametes in which some chromosomes are duplicated. Fusion of such gametes produces individuals which possess one or more extra chromosomes.

It would appear, however, that the occurrence of fragments seems to be very much more common in the meiotic cells than the occurrence of duplicated chromosomes. And contrary to all expectation we do not find these fragments getting lost but behaving like ordinary chromosomes. They even synapse and this would lead us to infer that the chromosomal fragments, however small, must contain some portion of the centromere, for otherwise they would get lost. Will it be too much to suppose that these fragments such as occur in the meiotic cells are the satellites of the C_1 and the C_2 chromosomes? Altogether there should be two C_1 and two C_2 satellites. We generally do not see more than four fragments. Occasionally we see five fragments (Figs. 18, 28, 31). This may be due to one of the fragments having divided again. The lower numbers may be due either to the elimination of some or the synapsis of these so that we get at MI two fragment bivalents. All the fragments may not synapse and that is why we get different numbers of fragments at MI. Union of a gamete with a fragment (from a C_1 chromosome) with another normal gamete will form an individual with 21 chromosomes in which there is only one C_1 chromosome, the other having fragmented. Similarly we get other chromosome numbers. It seems therefore likely that it is only plants in whose chromosome complements the C_1 and the C_2 chromosomes have been affected

that have arisen from such a course as described above. These are individuals that will show the same chromosome number throughout. The other method of alteration of chromosome number is by non-disjunction, and the union of gametes having different numbers. In these fragments may not be present but duplicated chromosomes.

The other cases do not show a constant number but will show different number in different portions of the same plant. Such numbers have presumably arisen through mitotic aberration, fragmentation of chromosomes other than the C_1 and C_2. And since such fragments may not be expected to include any centromere, they will get lost during mitosis or meiosis. And consequently only normal gametes may be formed.

Individual plants are being isolated for a more intensive study which it is expected will throw more light on this problem.

VI. Summary

A cytological examination of the diploid population of *Urginea indica* revealed the existence of chromosomal fragments in the somatic and meiotic cells. Variable number of fragments occur in the same individual. It is also suspected that there exist entire individuals having a definite number of fragments in addition to the usual diploid number (2n+1f; 2n+2f; 2n+3f and so on). Such individuals have not yet been isolated.

The behaviour of fragments in meiosis has been described. It is suggested that fragments from the C_1 and C_2 chromosomes would persist and the fusion of gametes having these fragments would result in individuals with varying number of these bodies. The fragments from the other chromosomes tend to be lost in mitosis and meiosis. An idiogram study of complements (21, 22, 23, and 24) having fragments of the latter kind has been presented.

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Studies in the Indian Scilleae
IV. The Cytology of triploid *Urginea indica* Kunth.

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I. Introduction

In a previous paper (Raghavan 1935) the somatic chromosomes of *Urginea indica* were studied in some detail, and the possible prevalence of triploidy was indicated. The study of *Urginea* had to be suspended for a time, and last summer we began an intensive study of the meiotic details. *Urginea* is a bulbous plant thriving in clayey soils around the University area. The most inconvenient thing about the genus is the very short duration of the flowering period. It flowers in the hot weather soon after a shower, March-April, and is confined to just over a fortnight. The scape is naked. Though the presence of triploidy was foreseen, the isolation of triploids and diploids from a heterogenous population under these conditions proved somewhat difficult. In the first cases, isolation was effected after careful examination of aceto-carmine smears of pollen mother cells. It was found after this preliminary work, that macroscopic isolation was also possible, as it was discovered that the scapes of diploids were very much shorter than those of the triploids. There was also a certain amount of correlation in the matter of the size of the bulb, but upon this much credence

could not be placed, as disclosed by later cytological verification. But the scapes are present for such a brief space of time that all the work connected with isolation, meiosis and measurement of rate of growth of the scape had to be compressed within that period. The cytological technique adopted does not call for any special mention. Root tips were fixed in Navashin's fluid as also anthers of the right stage of development, after prefixation in Carnoy. For smears which were made very commonly, Belling's Navashin was employed and for staining Newton's Iodine Gentian-violet was used almost exclusively.

II. Comparison of Diploid and Triploid

The most characteristic difference between the two is confined to the height of the scape. Fig. 1 gives a comparative idea of the size of the diploid and the triploid scapes. To a certain extent the size of the bulb is also bigger in the triploid than in the diploid, but this is a character which cannot be relied upon for purposes of isolation. The following figures give readings of the heights of triploid scapes from a dozen plants, and the corresponding values for diploids are also given alongside.

1	2	3	4	5	6	7	8	9	10	11	12	(ht. in feet)
3'.1"	3'.2"	2'.1"	3'.1"	3'.8"	3'.8"	3'.6"	3'.4"	3'.4"	3'.6"	3'.8"	3'.10"	Triploid
1.1	2.1	2.0	1.1	1.9	1.5	2.3	1.9	2.3	1.7	1.9	1.1	Diploid

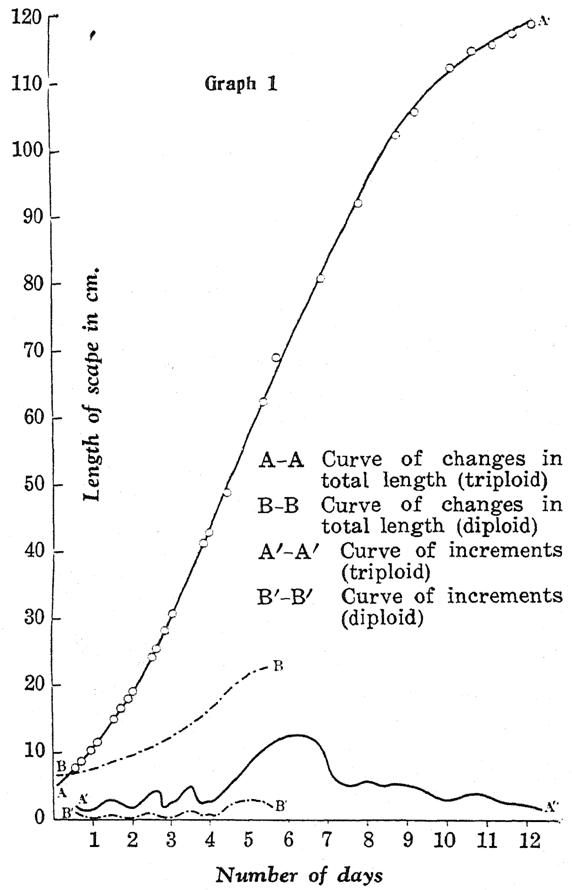
Readings taken of the lengths of pedicels did not reveal much of a correlation in this direction. The average length of a triploid leaf is about 8 inches, breadth 0.75 to 1 inch. In the diploid, the corresponding figures are 5 inches and 0.5 inch.

Some interesting observations were made in respect of the changes in total length and the rate of growth of the floral scapes. The readings taken are plotted in the form of curves in Graph 1. Curve A–A is that of the triploid. It is the curve of changes in total length establishing a relation between absolute elongation and suitable time intervals. It will be seen from the curve that the full length of the scape is reached on or about the 12th day. It may also be mentioned that pollen mother cells at first and second divisions are generally got from scapes which are hardly 15–20 cms in height, the full size of triploid scape being about 100–120 cms.

Curve B–B is the corresponding curve of the diploid. Unfortunately as will be seen from the curve, the plant from which these readings were being taken, perished after about the 6th day, and therefore observations could not be continued to the end of the

12th day. In both, however, the total length of the section is of the letter S-type. This is in accordance with the shape of ordinary curves of changes in total length (Maximov 1930).

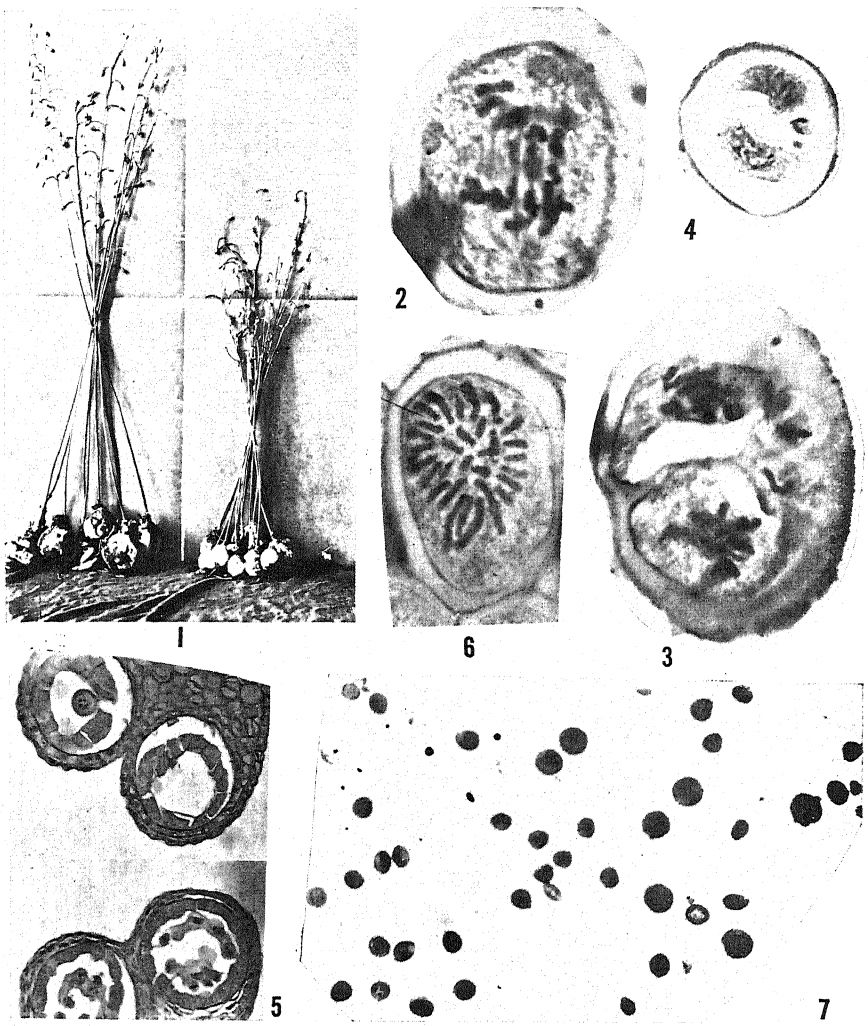
The other two curves, A′–A′ and B′–B′, are the curves of increments, the changes in the rate of growth at suitable time intervals. Barring the smaller fluctuations, rises and falls, the curves have almost a symmetrical aspect, with rather a prolonged maximum. This conforms to Sachs' law of the grand period of growth, by which every organ and each definite region of a developing organ at first grows slowly, then accelerates, reaches its maximum rate and finally settles down. It can be seen that curves, A′–A′ and B′–B′ run almost parallel to one another, though unfortunately observations beyond the 6th day could not be made in the case of the diploids.



The noteworthy things to be deduced from the curves are that firstly the curve of increments and the curve of changes in total length, both conform to their usual respective patterns and secondly, in both the curves, the curve of the diploid conforms to a subordinate position to that of the triploid. Especially is this significant in connection with the rate of growth as disclosed by the curve of increments; it is lower in the diploid than in the triploid.

So far as the time of flowering is concerned, there is no discrepancy between the diploid and the triploid. All of them flower

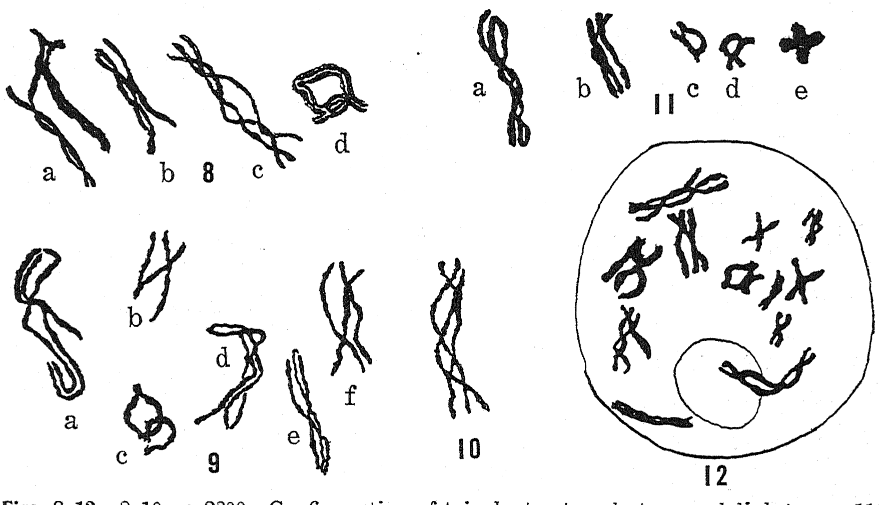
almost simultaneously. Besides these, measurements of homologous cells of diploids and triploids revealed a positive correlation. Fig. 5 is a photomicrograph of another loculus of a diploid and triploid, side by side. The difference in size of the pollen mother cells is evident. Fig. 6 is a photomicrograph of a somatic metaphase plate of a triploid.



Figs. 1–7. (Photographs.) 1. Diploid and triploid individuals to show comparative height of scapes. 2. Anaphase I. Irregular disjunction due to some of the univalents lagging. 3. Formation of the microcells by two eliminated chromosomes; same as Fig. 35. 4. A single eliminated chromosome forming a microcell; same as Fig. 32. 5. Diploid and triploid anther sacs to show the comparative size of the respective p.m.c at first metaphase. 6. Somatic metaphase plate; triploid, 2n 30. 7. Polymorphic pollen grains.

III. Meiosis in the Triploid

Earlier stages of the pollen mother cells show the leptotene threads running parallel to one another in twos and threes, coming occasionally together to form thicker strands. Regular pairing is initiated at zygotene when two of the threads are associated along a part of their length, while the third thread is hardly affected (Figs. 8–10). It is noteworthy that all the three threads seldom meet at a point. Sometimes, however, there appears a condition when a pair appears to be touched at one point by a third thread (Fig. 9a). This is a result of two chiasmata meeting and a careful examination reveals that though an exchange of partners takes place among the three chromosomes at the point of contact, the thread that leaves the association is not the same one that entered into it. Association can begin at many different points along the length of the chromosomes. Occasionally it begins at the ends.



Figs. 8–12. 8–10; ×2200. Configuration of trivalents at pachytene and diplotene. 11. Trivalents at different stages showing a reduction in the number of chiasmata. 11e shows a taivalent of the cruciform type. 12. Entire p.m.c at early diakinesis showing six bivalents and six trivalents.

Longitudinal contraction and consequent thickening is more marked in the double threads, for these appear to be shorter than the corresponding portions of single threads. It is on account of this consequential contraction that paired threads in the same nucleus, having paired at different times, appear of different thicknesses. As a result of this contraction, the nucleus becomes smaller and the mass of chromosomes denser. The third chromosome of the trivalents which was so far lying more or less parallel to the paired

threads without much of a contact, becomes more intimate (Figs. 8d and 11d). Chiasmata become more prominent and frequent and as already pointed out, at certain points all the chromosomes of a trivalent appear equally associated. But this appearance is due to the fact that the short distance along which the third chromosome appears to be in contact with the other two is in reality lying at a different focus and partially concealed by the pair.

Figs. 11a–e show the configurations of trivalents at middle diplotene, late diplotene and diakinesis. Obviously there is a reduction in the number of chiasmata due to terminalization, as contraction advances. Fig. 12 is that of an entire pollen mother cell showing six trivalents and six bivalents. Though a large number of cells were examined, associations of more than three chromosomes were never found, and only occasionally were the thirty chromosomes in the form of ten trivalents (Figs. 13 and 24). Associations were only partial and the chromosomes were in the forms of varying numbers of trivalents, bivalents and univalents. On account of this the number of chromosomal bodies seen at Metaphase I is variable (Figs. 19–22). Figs. 13–18 show p.m.c. at diakinesis with variable numbers of gemini. It must, however, be said that the number of univalents occurring is comparatively low. In side views of metaphases (Figs. 23) the univalents generally lie more or less outside the equatorial plate. In 36 metaphase groups examined the following are the numbers of univalents observed:—

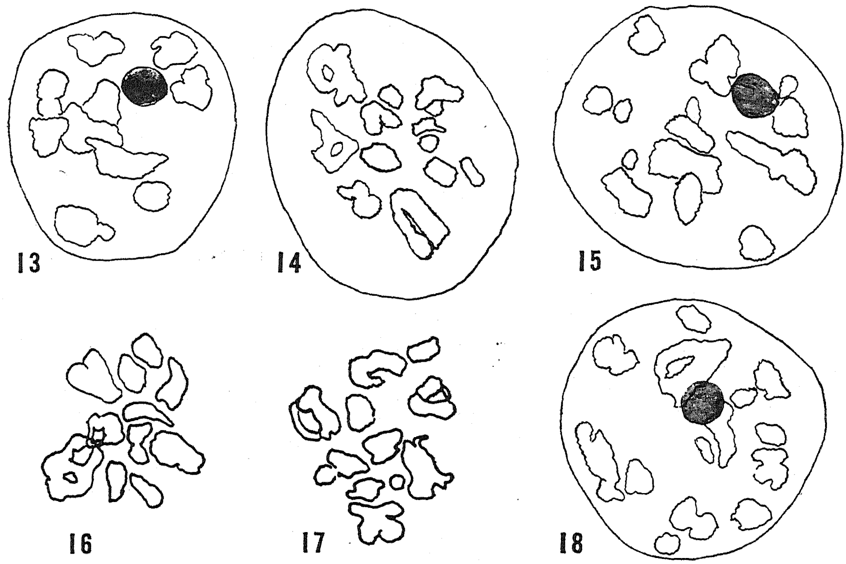
No. of univalents	0	1	2	3	4
,, cases	11	10	6	6	3

On an average there were 1.4 univalents per cell. As the basic number of the species is ten, it may be said that about 86% of the chromosomes occur as trivalents in the triploid. Though rarely one meets with univalents more than three, yet 1–3 per cell seems to be the common number. These figures indicate the relatively low number of univalents and the high frequency of trivalent formation.

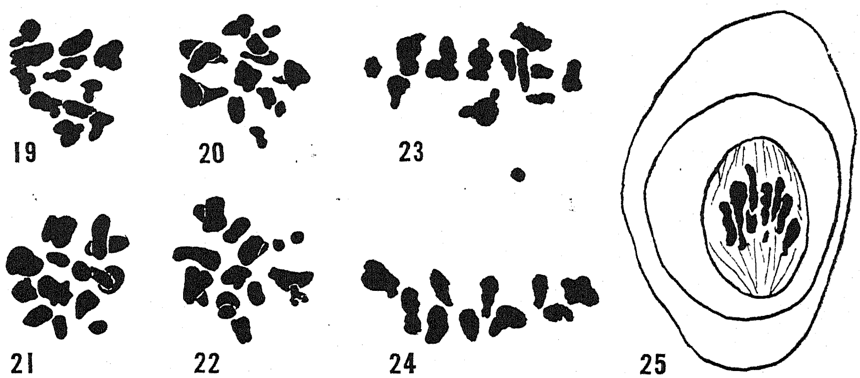
Fig. 18 shows late diakinesis nucleus with fourteen bodies as five trivalents, six bivalents and three univalents. Fig. 15 shows thirteen bodies in the form of 7_{iii}, 3_{ii}, 3_{i}. Figs. 19–22 are those of first metaphase plate showing, 12, 13, 14 and 13 bodies respectively. The most frequent number, however, is eleven.

First and second divisions show a number of irregularities. At first anaphase, a varying number of lagging chromosomes are observed (Figs. 2 and 42). In most cases these are univalents. In a few cases the trivalents are late in disjoining their chromosomes and are consequently stretched out on the spindle (Fig. 2).

The univalents have a tendency to split. Varying numbers of chromosomes are eliminated which form invariably a number of micronuclei. Fig. 32 shows interphase nuclei with an entire chromosome eliminated and formed into a distinct micronucleus. Fig. 4 is a photomicrograph of the same. Fig. 48 shows also interphase nuclei after the first division, wherein could be seen that not only are



Figs. 13–18; Entire p.m.c at middle diakinesis showing varying numbers of gemini ×1650. 13. Ten trivalents. 14. Twelve bodies. 15. Thirteen bodies; (7_{iii}, 3_{ii} and 3_{i}). 16. Eleven bodies; note the interlocking between two trivalents. 17. Twelve bodies. 18. Fourteen bodies; (5_{iii}, 6_{ii} and 3_{i}).



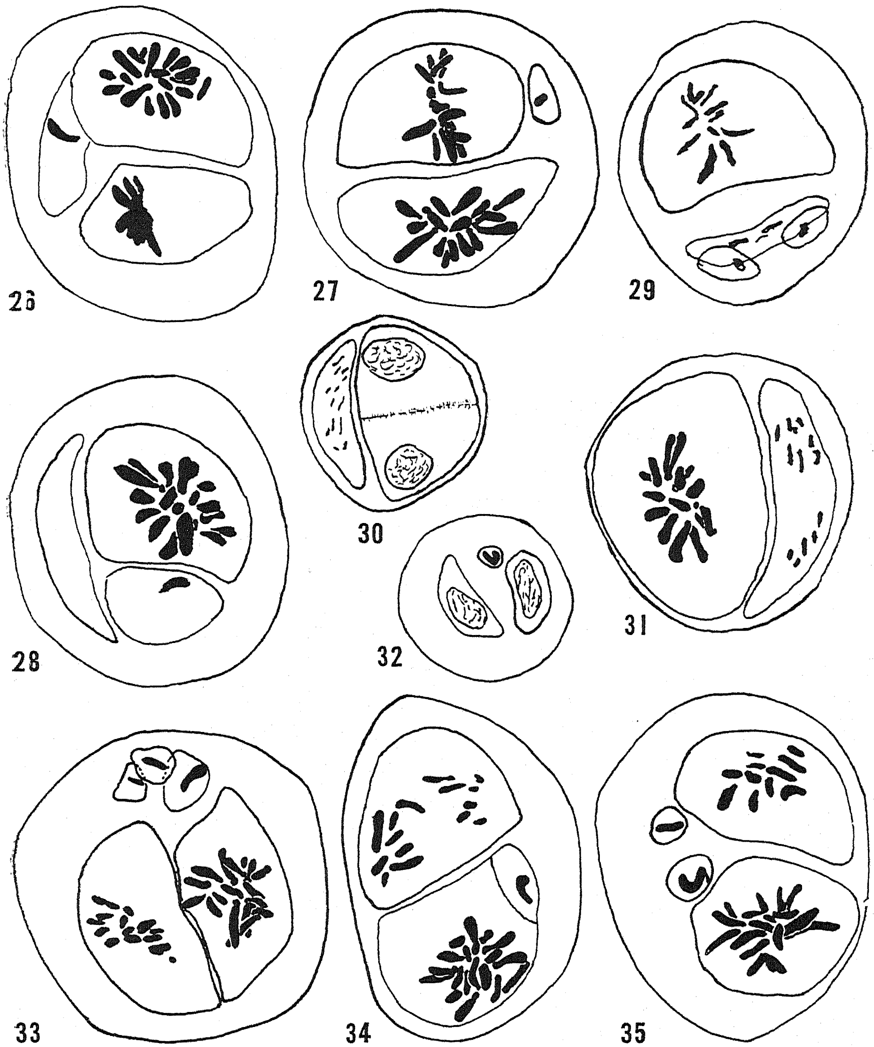
Figs. 19–25. ×1100. 19–22. First metaphase (polar view) showing 12, 13, 14 and 13 bodies respectively. In 22 the two univalents can be seen very clearly. 23. First metaphase side view showing thirteen bodies; the univalents are lying outside the spindle. 24. Side view, MI, a case of 10 trivalents. 25. P.m.c in MI, with persistent nuclear membrane.

the two daughter nuclei very unequal in size but also the two daughter cells organized. Unequal daughter nuclei have been recorded in the meiotic divisions of *Crepis* hybrids (Müntzing 1934). The formation of unequal cells seems to be a very widely prevalent phenomenon so far as triploid *Urginea* is concerned. In some cases one of the two cells resulting from the first division does not take part in the division, so that what may be termed triads are formed ultimately. In such cases the disparity in the size of the cells is very striking. Fig. 30 shows one of the two cells crescent-shaped, smaller and obviously not taking part in the next division while the other is much bigger and has almost completed the first division. Similarly in Fig. 31 the bigger cell is in second metaphase, while the smaller crescent shaped cell shows in it the chromosomes in a scattered manner. Darlington (1937) has figured such cells as occurring in triploids and he considers the scattered arrangement of the chromosomes as due to a kind of non-pairing. Fig. 28 is that of a three-celled pollen mother cell. It is doubtful if it is quite accurate to call these triads, since the bigger cell is really a dyad, not having undergone the second division, while the other two are ordinary tetrads.

The formation of micronuclei seems to be a frequent phenomenon in triploid meiosis. Dark (1932) has recorded the formation of these in the pollen mother cell divisions of triploid *Hemerocallis*. This, according to him results in the production of 'irregular tetrads', containing varying numbers of pollen grain. According to him, these micronuclei arise from metaphase univalents being situated too far from the equatorial plate to be included with the other chromosomes in the separation at anaphase. Takenaka (1929) says that these arise from stray chromosomes. He has also observed them undergoing longitudinal splitting, giving rise to small daughter nuclei. Therefore a number of daughter nuclei varying in number and size may be formed in a pollen mother cell. Figs. 26–35 show a number of such cases. It is seen, however, that these stray chromosomes are very often cut off by a wall, much in the same manner as a normal pollen tetrad is (Fig. 50). In the light of this observation and of the frequent occurrence of such cases to be described presently, it seems more appropriate to call these microcells rather than micronuclei. In Fig. 3 can be seen micronuclei surrounded by a definite wall constituting what has been called a microcell (cf. Figs. 26, 27, 28).

There is another kind of abnormality that is met with occasionally. Pollen mother cells at early prophase were found to contain two normal-sized nuclei instead of one (Fig. 57). Some-

times one of the nuclei was large, the other very small (Fig. 56). The most interesting thing about these nuclei, whether large and equal-sized or of unequal size is that they behave independently of



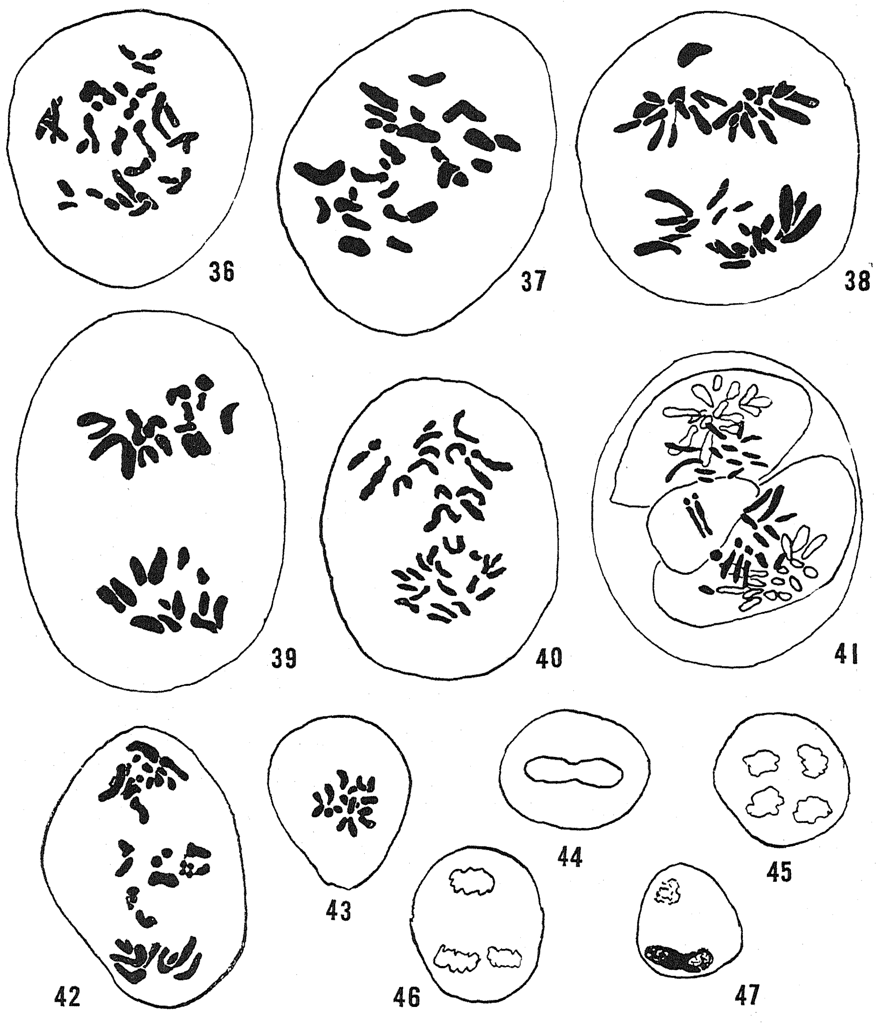
Figs. 26–35. ×1100. 26. MII; formation of a microcell by a process of constriction by an eliminated chromosome; 19 chromosomes can be counted in one cell. 27. Same as above; one of the cells shows 16 chromosomes. 28. A case of unequal cell formation. 29. do; three small cells formed by stray chromosomes and one large cell. 30. The large cell in telophase of second division while the small crescent-shaped cell is apparently not taking part in the second division. 31. Non-simultaneous division; big cell in MII; the smaller cell in anaphase. 32. Same as Fig. 4. 33. Three eliminated chromosomes forming as many microcells. 34. Stray chromosomes being cut off by a wall from the bigger cell which is in second metaphase. 35. MII with two microcells and two large cells (11 17 2).

one another. Sometimes both of them are at the same stage of meiosis or of different stages (Fig. 58). As a result of this, if the two divisions are undergone we should get at the tetrad stage eight haploid pollen grains in the one with two normal-sized nuclei and in the other, four haploid and four small grains must result. Such were not, however observed. Presumably the second division is suppressed. That is why in Fig. 54 we see two big and two small nuclei. In Fig. 55, the two big and two small cells have been organised around the nuclei after the first division. If these nuclei did not behave independently, a doubling of the chromosomes would result. Polymorphic pollen grains do occur indicating the occurrence of polyploid pollen grains, but the origin of these is more due to the suppression of wall formation after the first meiotic division followed by the fusion of the Metaphase II nuclei than to the fusion of nuclei at the p.m.c. stage.

The origin of the small nuclei in the pollen mother cell is not quite clear. Presumably a pollen mother cell with two normal-sized nuclei arose by suppression of wall formation at the premeiotic mitosis. Perhaps abnormal spindle behaviour such as failure of coordination of one of the poles at the premeiotic mitosis may have played a part in producing a pollen mother cell with a large and a small nuclei. Binucleate pollen mother cells have been observed by some workers. Upcott (1939) found pollen mother cells of this kind in the triploid *Tulipa*, "Pink beauty". Crane and Thomas (1939) found similar cells in the pears (fertility $2x$), at pachytene, metaphase and diakinesis.

Fig. 43 is that of first anaphase with 16 chromosomes in one pole. This irregularity in the matter of disjunction is accentuated by the formation of microcells by the eliminated chromosomes mostly univalents. For example in Fig. 39, we see as each pole 13; presumably the eliminated chromosomes have been lost. Fig. 40 looks very much like a case of asymmetric anaphase. In Fig. 29 we see one big cell and three microcells. In another case, Fig. 49, we see three cells two big and of equal size, and the other small, the latter being formed around two eliminated chromosomes. Of the two bigger cells, the upper one shows fifteen chromosomes, while the other shows almost double the number of chromosomes, about 30. Both these are in second metaphase. The interpretation of this is that the lower cell is to be regarded as a restitution nucleus. Failure of the second anaphasic separation must have been the cause of this. In the other cell the chromosomes are in second metaphase and whether it will undergo the regular homotypic division cannot be said with certainty. In any case this interpreta-

tion involves the supposition that the divisions in the two cells are non-simultaneous. This, however, seems to be not infrequent, as can be seen in Fig. 34 where one of the dyads is in second metaphase,



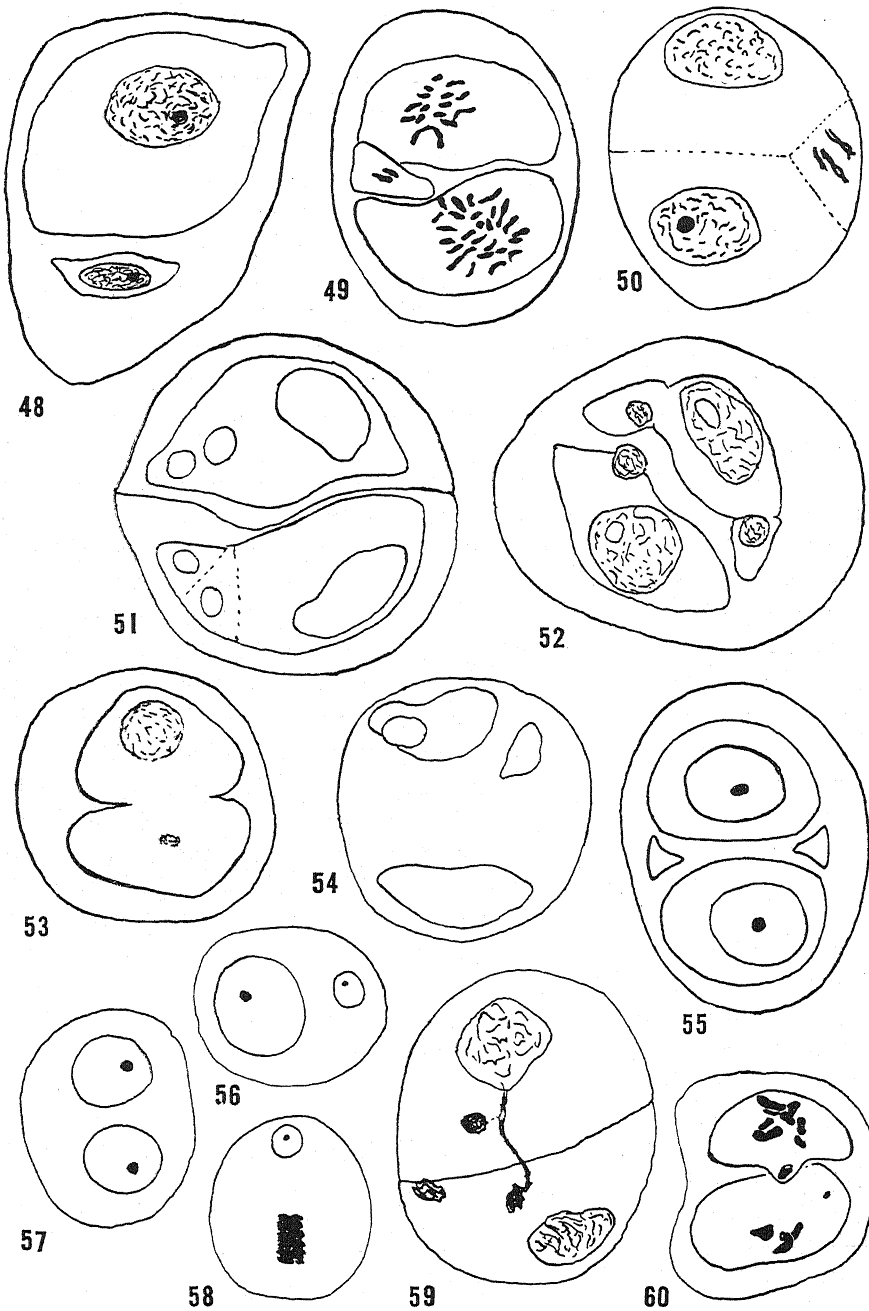
Figs. 36–47. ×1100. 36. P.m.c with diploid number of chromosomes (restitution nucleus). 37. MII showing no wall formation; there is a tendency for the fusion of the two second metaphase plates. 38. AII where all the four poles are in the same plane and exhibiting a tendency to fuse there being no cross wall formation at all. 39. AI showing no cross wall formation (13 13). The other chromosomes have been lost. 40. Asymmetric anaphase AI. 41. AII; 15/15 on the right; 14/13 on the left and a smaller cell with two chromosomes presumably derived from the longitudinal division of a single one. 42. AI with a lagging chromosome. The univalents can be seen dividing. 43. AI polar view, showing 16 chromosomes in one pole. ×750. 44. Ameiosis, the chromatin mass in constriction presumably before division into two. ×420. 45. Four masses derived by this process of fragmentation. ×420. 46-47. Earlier stages of the same. ×420.

while the other is in second anaphase; there is also an eliminated chromosome forming a microcell.

There is another abnormality worth recording and that is the frequent persistence of the nuclear membrane even at first metaphase (Fig. 25). The formation of supernumerary cells caused by eliminated chromosomes has been mentioned already. For example in Fig. 33 which is a pollen mother cell in second metaphase, there are three microcells formed by as many eliminated chromosomes. There are thus already five cells even before the second division has taken place. If the homotypic division also takes place in which all the cells take part, then there will result, ten cells, four large and six small. This, however, is improbable for seldom have we seen so many supernumerary cells. The most usual procedure is for these cells to take no further part in division and organize themselves as daughter cells. In Fig. 52 the chromosomes have resolved themselves into the resting nuclear condition and in this state they exist in the cells. In some cases these microcells also exist with their micronuclei. The method by which such microcells are cut off calls for some mention. It may be a process of constriction (Fig. 52), or by the intercalation of a plate-like wall. In Fig. 51 two such cells are being separated from the big dyad while two may take place later. In some cases this cytokinesis is effected so as to separate microcells from the others at a comparatively early stage. In Fig. 50 three chromosomes are seen being cut off by a wall formation.

While normally a cross wall is formed after the first division, very frequently cases are found where there is complete failure of wall formation. Fig. 37 is that of second metaphase with no cross wall. In this can be detected a tendency towards a fusion of the two metaphase plates. The full 30 chromosomes could not, however, be counted. Presumably this is due to the elimination of a few chromosomes. This is unmistakably evident in the next Fig. 36 where the full diploid number could be counted. Such a fusion of metaphase plates is a common method of forming unreduced gametes and has been observed among others, in *Solanum tuberosum* (Müntzing, 1932). The next Fig. 38 shows second anaphase with the four polar groups lying close together and parallel. This is undoubtedly a case of a fusion of homotypic spindles.

In one case we found a chromosome or an elongated mass of chromatin delayed in separation and this was cut through by the formation of the cell wall (Fig. 59). Such a phenomenon was also observed by Krishnaswami (1939) in haploid *Triticum vulgare*. Sometimes the wall instead of cutting through the chromosome as



Figs. 48–60. ×1100. 48. Unequal cell formation at the end of first division. 49. MII; on the lower cell the full diploid complements are seen owing to failure of anaphasic separation. 50. Interphase and three stray chromosomes being cut off by a wall by the usual cytokinesis. 51. Micronuclei being cut off as cells by wall formation. 52. Microcells cut off by constriction. 53. Abortive cross wall

mentioned above, bends round the chromosome thus protruding into the other cell (Fig. 60).

In a few cells cross wall formation after the first division is abortive (Fig. 53). Fig. 41 is that of second anaphase where the number of chromosomes in each pole can be counted more or less clearly. On the right could be seen 15/15 while towards the left 14/13 could be counted. And two chromosomes have organized themselves into a microcell. The question is whether these represent two eliminated chromosomes or the divided halves of a single eliminated chromosome. The probability is that it conforms to the latter interpretation. It is likely that since we find 14/13 in the poles of the left handed cell, one of the chromosomes was eliminated during first division and that this stray chromosome also underwent the second division, even as the normals, and then organized themselves into a microcell. Their closely paired position is in support of this interpretation. Takenaka (1929) who has also seen such stray chromosomes forming micronuclei thinks that these undergo longitudinal splitting.

Sometimes a continuous chromatin thread extending from one interphase nucleus to the other. It is presumed that this results from a number of small eliminated or stray chromosomes being placed almost end to end, and having undergone a process of resolution as the normal chromosomes do at the poles. Takenaka's (1929) Fig. 29 represents seven small daughter nuclei as a result of irregularity of meiosis. If the chromatin threads of these had lengthened out in a process of resolution then we may get a configuration as described above.

On account of these abnormalities pollen viability is very low, and the microspores formed are of different sizes. This polymorphism is best seen in Fig. 7 which is a photomicrograph of a number of these pollen grains. The relative size and variation of the pollen is given in an analysed form in the accompanying table.

	Pollen diameter						Total no. & average
Triploid	6	5	4	3	1.5	units	3.86
	2	25	81	45	2	no. of grains	155

formation after the first division. 54. Binucleate p.m.c; the nuclei by their independent division have formed 2 large nuclei and two small nuclei. 55. do, the two smaller and the two bigger nuclei have organised themselves into as many cells; presumably the second division is suppressed. 56. Binucleate p.m.c; one large and one small nucleus. 57. Same as above but with equal sized nuclei. 58. do, the smaller nucleus in the resting condition, the larger in the metaphase. 59. The chromatin threads being cut across by wall formation. Note also the presence of micronuclei. 60. The cross wall has bent around a lagging chromosome.

IV. Ameiosis

Very often the nuclear mass suffers a sort of constriction by which it is divided into usually unequal nuclei. This takes the place of the first division occasionally. But more commonly and very frequently such divisions take the place of the second division. The former since it takes the place of the heterotypic division, can be termed ameiosis, and the latter, since it is a substitute for the homotypic division, a sort of amitosis. Of the former type Fig. 53 is an example where two unequal nuclei or two masses of chromatin material have presumably been formed by the splitting of an original single mass. The abortive wall formation is in support of this interpretation. In Fig. 44 the chromatin mass may be seen in a process of constriction. In the second division, the splitting of the chromatin mass at one or both the poles is of common occurrence. In Fig. 47 one of the two chromatin masses is in the process of such a division as could be seen from the constricted nature of the mass. In Figs. 46 while the clump of chromatin threads at one pole has split up into two the other has remained unsplit. In another, Fig. 45, splitting has taken place in both the poles. Such irregularities are probably the result of genetic unbalance in the cells and such cells ultimately result in the formation of abortive gametes. This can very well be seen in Fig. 7.

V. Restitution Nucleus

In a few cells there was some lack of regularity in respect of wall formation at the end of first division and not infrequently this resulted in the formation of restitution nuclei. These are of normal shape and size. Fig. 36 shows a pollen mother cell with thirty chromosomes. Obviously there has been more or less complete lack of pairing coupled with failure of wall formation. This usually results from the failure of the chromosomes to reach an equatorial position at first division. Such a complete absence of pairing was extremely rare. Whether the second division is normal in such a restitution nucleus cannot be said for we have not observed any cell other than the one described above.

A related phenomenon was observed in the second division also, the failure of the chromosomes to move to the poles. Undoubtedly such cells may form diploid pollen grains. That polymorphism in pollen grain size occurs has already been recorded. The actual number of chromosomes in the various-sized microspores could not be determined. But it is very likely that the giant pollen grains represent polyploid gametes in whose formation one of the methods

has presumably been the occurrence of restitution nuclei.

The formation of restitution nuclei seems to be a variable phenomenon among triploids. Some like *Narcissus* (Nagao, 1929) show frequent formation of restitution nuclei, while others like *Zea mays* (McClintock, 1929) and *Solanum* (Lesley, 1928) show practically no restitution nucleus formation. Again Ramanujam (1937 a and 1937 b) has found restitution nucleus formation in interspecific hybrids of *Oryza* but could see none in an autotriploid of the same plant.

VI. Discussion

a) Intraspecific chromosome races

Most plant species are characterized by a specific chromosome number. A number of species are known having different chromosome numbers. The number of such intraspecific chromosome races is increasing. Müntzing (1936) has studied the characteristics of fifty eight cases of intraspecific races. From his observations the following stand forth prominently: Intraspecific chromosome races are practically always morphologically different from each other. The great majority of the species consist of races that are quantitatively different. This is only natural since qualitative differences may raise the deviating types to the rank of a new species. And the relationship is one of marked positive correlation between chromosome number and the gigas characters studied. The races having the high chromosome number tend to be vegetatively more robust than those with the lower chromosome number.

The readings taken in this study of the length of leaves, and scape in diploid and triploid *Urginea* confirm this positive correlation that Müntzing has discovered. Comparison of cell size has also conformed to this positive correlation. Being a triploid, the plant does not set seeds and therefore there is no question of the comparison of capsules and seeds. It is presumed that the greater size of the cells is caused by the increased chromosome number.

Though there is this strong positive correlation between morphology and chromosome number, there would appear to be an optimum for chromosome increase beyond which the individuals become less vigorous. The position of this optimum seems to be different for different species. In the mosses (Wettstein, 1927), this limit is reached by the tetravalent races, which in higher plants would correspond to the octoploid condition. Babcock and Navashin (1930) found that the autotetraploid *Crepis capillaris* is inferior in vigour to the diploid. Similarly in *Primula obconica* (Sansome and Philip, 1932).

Navashin (1929) found that his triploid *Crepis* plants were distinguished by their slow but robust growth compared to the diploids. Also the triploids started to bloom about a month later than the diploid sister plants, although planted at the same time and grown under exactly the same conditions. In our material of *Urginea indica*, however, we do not find anything to confirm these observations. True it is that in the species in question it is difficult to calculate the rate of growth of the plant as the scape comes out when the plant is bare of foliage; and the readings we have presented relate to the rate of growth of the scape. As far as we could see it is a very fast growing organ and this much can be said with the data on hand, namely that so far as the growth rate of the floral scape is concerned, it is more rapid in the triploid than in the diploid and the former attains a greater height also. Enough observations have not been made to generalize in respect of the vegetative portions, for example the leaves also. In regard to the time of flowering there is no discrepancy between the triploid and the diploid. They grow side by side under exactly identical conditions and the prevalence of triploidy is wide spread. In certain localities, nearly 40% of the population are triploids. We have observed that there is no marked difference, neither undue lateness nor undue quickness, in the matter of the coming into bloom of the triploids as compared to the diploids. And since they bloom simultaneously and since the scape of the triploid is much higher than that of the diploid, it is only natural that the rate of growth of the former must be very much faster than that of the diploid. The flowering period of *Urginea* is, under natural conditions a very brief period, something less than a fortnight in the hot weather generally in the months of March-April, immediately after a shower, and we have not so far seen these plants in bloom after this brief period. It cannot be therefore that the triploids started flowering a month or so later than the diploid plants.

There are other cases known besides *Crepis* which demonstrate a retardation in the rate of development. Collins (1933) reported that triploid varieties of *Ananas* mature more slowly than diploids. Similarly the gigas form of *Cleome spinosa* is slower than the normal plants in point of flowering (Ufer, 1927).

One case, however, comparable to the present is that of *Petunia violacea* (Kostoff and Kendall, 1931), where it was reported that an autotetraploid individual grew more rapidly than its diploid sister plants.

A number of important conclusions have been drawn from this observation. Firstly there is a correlation between high chro-

mosome number and the perennial habit. This connection, it is presumed, is due to a correlation between chromosome number and the rate of cell division, which results in a retardation of the rate of development. And the connection between the retarded development of experimental autopolyploids and the high chromosome numbers of the wild perennial species and other similar relationships have led Müntzing (1936) to propound an important theory that the perennial species have originated from annual types with lower chromosome numbers. How far this will be generally acceptable cannot be said, for to a certain extent it runs counter to the almost accepted theory of the evolution of the annual herbaceous forms from woody perennial forms. And the phenomenon of slower growth of polyploid forms compared to the diploid upon which this theory is built does not seem to be indisputably universal as the present case as well as the case of *Petunia violacea* (Kostoff and Kendall, 1931) show data to the contrary.

b) The possible origin of the triploid

It was Gates (1908) that first discovered triploidy in *Oenothera*. In a hybrid population of a cross between *Oenothera lata* and *Oe. Lamarkiana*, he observed a plant with a diploid number of 21 chromosomes. And this he believed to have resulted from hybridization between the two.

The autotriploids have three identical genoms (AAA) while the allotriploids may have genoms, AAB or ABC and so on. Of the many causes that may be responsible for the production of autotriploids, the more common are the fusion of a diploid gamete with a haploid gamete, and the phenomenon of dispermy. Gates (1924) was the first to suggest that triploid mutations may arise from a chance union of two sperm nuclei with a haploid egg. Cases of dispermy have been reported from time to time, one of the most recent being that of Rhoades (1936). He has reported that his triploid *Zea mays* arose from the simultaneous fusion of two haploid sperm nuclei with a haploid egg. The fusion of a diploid with a haploid gamete seems to be more frequently responsible for the production of the autotriploid. In this again the diploid eggs are reported to be more functional than the diploid male gametes. A few cases of diploid male gametes functioning in this direction are also known. So far as the origin of triploidy in *Urginea* is concerned we have firstly to remember that the pollen grains of the diploid are all of the same size while those of the triploid, Fig. 7, exhibit polymorphism. Viability in the latter is very low while in the former spindle-shaped

generative cells can be clearly distinguished. Only in a few pollen grains could the chromosome number be counted. It may not, however, be unreasonable to presume that in the diploids, polyploid male gametes do not occur. This possibility having been ruled out, the other alternative is the formation of diploid eggs. We have examined a number of preparation of ovules and we have not found any sign of diploid egg formation. But this need not rule out this possibility completely, as the frequency of the occurrence of such cases might be extremely low and we cannot claim to have examined sufficient number of preparations to assert its absence. Dispermy would appear to be a possible method, but for this we have no data at present. We tried to grow the pollen tubes in cultures to see if there was any tendency for the fusion of the male gametes and though tubes were put out at a concentration of 25% (Wulff and Raghavan, 1937) the bursting was so extensive, that study in this direction was rendered impossible.

Another fact may also be well remembered in this connection. It has been observed that one or two roots of certain diploid plants are wholly tetraploid. Hollingshead (1928) found tetraploidy in the roots of two different plants of *Crepis*. One a *Crepis biennis* × *C. setosa* hybrid derivative had one root partially tetraploid. The other, a plant of *C. Bureniana* had two roots wholly tetraploid. The present case would correspond to that of *Crepis Bureniana*. It cannot be said with certainty as to the origin of these tetraploid roots. Presumably nuclear fusion has been responsible for this. The occurrence of these tetraploid roots in the same plant indicates that a part of the central cylinder from which branches arise had become tetraploid.

Whatever the method of origin, the frequent occurrence of tetraploidy in somatic tissues throws some light on the mode of origin of diploid gametes. Presumably under these conditions a small portion of the shoot is affected and a portion of the gametes may be diploid. Again the frequent occurrence of somatic tetraploidy has a bearing upon the origin of tetraploids and tetraploid hybrids. *Primula Kewensis* arose as a bud sport from a F_1 hybrid of *Primula verticillata* and *P. floribunda*. The bud sport arose by syndiploidy or the doubling of the somatic chromosomes. The somatic chromosome number of *Primula Kewensis* is the sum of the diploid chromosome numbers of the parent species.

We have not found any diploid gametes nor signs of them. And the question of a tetraploid portion of the shoot which may be expected to bear the diploid gametes is not tenable as the aerial portion of the plant consists only of a few leaves and there is only

a single scape. It may be that in the past crossing between the diploids and tetraploids did take place resulting in the establishment of a triploid race. But how does it happen that there is as yet no trace of the tetraploid parent? Unless it be that they have been exterminated outright in the competition. Examination of *Urginea* from all localities of south India is being made and the existence of a tetraploid race may be revealed.

In the meantime the case of a tomato (Huskins, 1934) where the triploid arose from a diploid stock somatically may be remembered in connection with the origin of triploid *Urginea*. Of course it is very difficult to explain and involves the presumption of the formation of a hexaploid cell and somatic reduction of this. The only circumstance that makes this merit any consideration is the wide prevalence of somatic pairing which has already been recorded by Raghavan (1935).

c) Triploidy and sterility

That triploids are generally sterile is a common feature. No seeds are borne by these triploid *Urginea*. From the very nature of the irregular meiosis functionally fertile pollen grains are of rare occurrence. This abnormality of pollen grain produced by the irregular meiotic divisions is largely responsible for the sterility of these triploids.

Sections of ovules at different stages were taken and we find that there is extensive degeneration of embryo sac. While upto the linear tetrad formation there is normality, beyond this stage there is extensive irregularity resulting in varying degrees of degeneration. The precise cause of this degeneration could not be investigated into. Presumably it is consequential upon irregular mitosis as was demonstrated by Tin (1928) in *Hemerocallis fulva* and *H. citrina*. Triploidy is one of the main causes of sterility in plants as it leads to an abnormal distribution of chromosomes in the formation of gametic cells. This inevitably causes a disturbance in the normal quantitative relations of the genes, which is essential for fertility.

VII. Summary

The existence of triploidy in *Urginea indica,* was suspected some years ago by the senior author. Triploids were now isolated from a heterogenous wild population. The diploid and the triploid have been studied on the basis of comparative morphology. The existence of a positive correlation between gigas characters and chromosome number has been confirmed.

A comparative study of the rate of growth of the floral scape has been made of the diploid and the triploid, and it was revealed that it is higher in the triploid than in the diploid. No discrepency was observed in respect of the time of flowering of the triploid and diploid. Both flower almost simultaneously. This is not in consonance with a few observed cases of retarded growth, exhibited by polyploid forms. The question is raised whether conclusions arrived at on the basis of the slower growth rate etc., of polyploids are to be accepted as universally true in the face of data, such as are presented in this paper and a few others, where there is no evidence for a slower rate of growth in the polyploids as compared with the diploid forms.

Meiosis in the triploid is described in detail. All the irregularities are described, like the elimination of chromosomes, formation of micronuclei and microcells, ameiosis, formation of restitution nucleus etc. The occurrence of unequal nuclei in pollen mother cells and their independent behaviour leading to the formation of tetrads of unequal size are also described. The possible origin of the triploid is also discussed in the light of the data on hand.

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Lebendbeobachtungen über die Einwirkung des Colchicins auf die Mitose, insbesondere über die Frage der Spindelfigur [1)]

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(Mit 48 Textfiguren)

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Einleitung

Der Mechanismus der Polyploidbildung durch das Colchicin wurde bei verschiedenen Materialien bereits von vielen Cytologen untersucht. Durch die Ergebnisse dieser Untersuchungen ist man heute zum Schluß gekommen, daß die Verdoppelung der Chromosomenzahl einerseits auf der Durchführung der Chromosomenteilung und andererseits auf dem Zurückhalten der Spindeltätigkeit beruht. Viele Forscher beschäftigten sich mit der Erklärung des Verhaltens der Chromosomen in der C-Mitose[2)] und mit derjenigen der feinsten Bilder einzelner fixierter Präparate, aber sie beachteten dabei kaum das Verhalten der Spindelfigur und ihre Veränderungen in ihren aufeinanderfolgenden Teilungsstadien. Viele von den Forschern nehmen an, daß die Spindelfigur unter der Einwirkung des Colchicins verschwindet. Neuerdings bemerkte SHIMAMURA (1939) einen Rest der Spindelsubstanz bei einer C-Mitose von *Allium cepa*. Er nahm an, daß das peripherische Verlegen der Halbchromosomen auf dem Zurückbleiben der degenerierten Spindelsubstanzen im Zentrum der Zelle beruht.

Da die Chromonemata, die Chromosomen, die Karyolymphe, das Atraktoplasma, das Atraktosom und der Phragmoplast—alle diese Teilungselemente teils stofflich, teils morphologisch miteinander in Beziehung stehen, ist es nicht ganz befriedigend, das Zustandekommen der C-Mitose lediglich als Folge der Untätigkeit der Spindelfigur hinzunehmen; man muß dabei auch die gegenseitigen Beziehungen dieser Teilungselemente in Betracht ziehen. Daher fühlte ich mich veranlaßt, Versuche über die Einwirkung des Colchicins auf

1) Contributions from the Divisions of Genetics and of Plant-Morphology, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 255.

2) Die Worte „C-Mitose“ und „C-Paarung“ wende ich hier in demselben Sinn an, wie LEVAN (1938) sie in der Colchicin-Behandlung bei den Keimwurzeln von *Allium cepa* benutzt hat; nach LEVAN nennt man alle modifizierten Arten der Mitose, die durch Colchicin-Behandlungen induziert wurden, kurz C-Mitose.

die Mitose im lebenden Zustand der Zelle, mit besonderer Berücksichtigung nicht nur der Spindelfigur, sondern auch aller anderen Teilungselemente anzustellen.

In vorliegender Arbeit habe ich die Einwirkung des Colchicins auf die Mitose durch Lebendbeobachtung über die folgenden Fragestellungen nachgeprüft:

1. Durch Lebendbeobachtung werden festgestellt die Geschwindigkeit des Eindringens des Colchicins in die sich teilende Zelle und die Konzentrationsgrenze des Colchicins, dessen Einwirkung auf die Mitose sofort in Erscheinung tritt.
2. Durch Lebendbeobachtung wird die Verschiedenheit des Colchicin-Effekts auf die Mitose untersucht, der sich beim Beginn der Colchicin-Behandlung den Teilungsstadien entsprechend zeigt; es sind die Teilungsstadien mit der Spindelfigur, dieselben vor dem Auftreten der Spindelfigur und dieselben mit dem Phragmoplast.
3. Durch Lebendbeobachtung wird die Lebensfähigkeit der behandelten Zellen in der Colchicinlösung untersucht.

Material und Methode

Als Untersuchungsmaterial benutzte ich die Mitose der Staubfadenhaarzellen von *Tradescantia virginica* und auch die von *T. reflexa*. Das Staubfadenhaar, welches eine sich teilende Zelle enthält, wurde aus einer jungen Blütenknospe isoliert und im hängenden Tropfen in einer Feuchtkammer untersucht. Bei dem Gebrauch des Colchicins zur Mitose wandte ich folgende drei Methoden an.

1. Versuche mit einer Mikropipette: Eine Pipette, deren Innendurchmesser 20μ beträgt, ist mit einer 2-proz. Colchicinlösung angefüllt. Bei der Behandlung bringt man sie mit einer sich teilenden Zelle eine beliebige Zeitdauer in Berührung; die Lösung dringt durch Diffusion in die Zelle ein. Wenn es sich als nötig erweist, wird ein Druck auf die Pipette ausgeübt, wodurch die Teilungszelle von der Colchicinlösung bedeckt wird.

2. Versuche mit Medien verschiedener Konzentrationen des Colchicins: Als Untersuchungsmedien stellte ich 0,005-, 0,01-, 0,05-, 0,1- und 0,5-proz. Colchicinlösungen her. Zur Verdünnung der Colchicinlösungen nahm ich oft 2-proz. Rohrzuckerlösung. Diese verdünnten Lösungen wurden auf Kulturversuche der behandelten Zellen angewandt.

3. Versuche bei eingetauchten Blütenknospen: Gleich vielen Forschern untersuchte ich die Mitose bei den Staubfadenhaarzellen der jungen Blütenknospe, welche in eine stark verdünnte Colchicin-

lösung (0,004%) eingetaucht wurden,[1] wo sie einige Stunden lang liegen blieben.

Bei der Lebendbeobachtung, wie den optischen Bedingungen, der Kultur der behandelten Zellen und der Mikrophotographierung wandte ich dieselbe Methode an, wie in meinen früheren Arbeiten, daher wird die Beschreibung darüber hier weggelassen. Alle Mikrophotographien der Textfiguren sind in ca. 900-facher Vergrößerung reproduziert.

Untersuchungen

1. Versuche mit einer Mikropipette: Abweichend von Versuchen, die auf die künstliche Herstellung polyploider Pflanzen zielen, benötigen diese Versuche sofortiges Eindringen des Colchicins in die Zelle, um seine Wirkung auf einzelne Teilungsstadien durch die Lebendbeobachtung festzustellen. Hierfür wandte ich die Methode 1 an und konnte feststellen, daß die Wirkung des Colchicins auf die sich teilenden Zellen in 30 Sekunden oder in einer Minute nach der Behandlung auftritt. Die Lösung aktiviert zuerst das Zytoplasma und ruft dann die Veränderung des Atraktoplasmas, also die der ganzen Teilungsfiguren hervor. Das Colchicin, dessen Konzentration in der Zelle natürlich niedriger als die im Medium ist, wirkt nach dem Herausziehen der Pipette aus der Feuchtkammer noch lange Zeit nach, es diffundiert aber allmählich die Umgebung der behandelten Zellen und geht schließlich aus der Zelle ins Medium, da die Menge der Lösung, die durch die Pipette ins Medium eingedrungen ist, im Gegensatz zu der Mediumlösung auffallend gering ist. Einige Stunden nach der Behandlung sind die behandelten Zellen frei von Colchicin. Dann zeigen die Zellen Neigung sich vom gestörten Zustand zum normalen zurückzubilden, und die Spindelfigur stellt ihre Tätigkeit wieder her. Jedoch enden die Mitosen dabei gewöhnlich auf abnorme Weise nur selten auf normale.

Von den Experimenten, die durch die Methode 1 angestellt wurden, gebe ich hier ein Beispiel zu Protokoll (Fig. 1–6). Die behandelte Teilungsfigur befand sich in der Terminalzelle eines Staubfadenhaares von *Tradescantia virginica,* welches in seiner Zellenlänge dasjenige von *T. reflexa* übertrifft, und sich daher zum Versuche mit einer Mikropipette besonders eignet.

15.04[2] am 1. August 1939. Metakinese. Vor der Behandlung (Fig. 1).

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1) Die in die Colchicinlösung eingetauchten Blütenknospen von *Tradescantia reflexa* wurden mir von Herrn Dr. SHIMAMURA aus seinem Untersuchungsmaterial zur Verfügung gestellt. An dieser Stelle spreche ich ihm meinen verbindlichsten Dank aus.

2) 15.04 bedeutet 15 Uhr 4 Minuten; die Worte Uhr und Minute sind (wie auch in den folgenden Zeitangaben) weggelassen.

15.08. Man bringt eine Mikropipette mit einer 2-proz. Colchicinlösung mit der Basis der Zelle in Berührung und drückt die Pipette schwach, wodurch die Zellen ein wenig in der Lösung verbleiben. Drei Minuten nach der Behandlung zieht man die Pipette aus der Feuchtkammer heraus. Während dieses Verfahrens dringt die Colchicinlösung schon in die Zelle ein; das Zytoplasma entmischt sich und die Zytoplasmastränge nehmen an Zahl zu. In den Ruhezellen prägen sich sowohl die Zunahme der Zytoplasmastränge als auch die Bewegung derselben aus.

15.17. Die Granulen im Zytoplasma um das Atraktosom gangen jetzt lebhafte BROWNsche Bewegung an, ohne daß sich dort Zytoplasmaströmungen zeigen. Die Grenzfläche des Atraktosoms verändert sich allmählich, indem sie nicht so glatt bleibt wie sie früher war, wobei die Chromosomen etwas diffuse und blaß aussehen.

15.27. Das Atraktosom sieht blaß aus, und seine äußere Gestalt ändert sich bis zur Unförmlichkeit, doch behält es seine Grenzfläche bei (Fig. 2*a*).

15.58. Einzelne Chromosomen strecken sich aus und halten sich gerade.

16.40. Im erweiterten Atraktosom zerstreuen sich die Halbchromosomen in paralleler Lage liegend. Einige von ihnen entsprechen der C-Paarung bei den LEVAN'schen Versuchen (Fig. 3).

17.17. Zwei Stunden nach der Behandlung. Durch Diffusion fließt das Colchicin aus den Zellen ins Medium hinein und das Atraktoplasma, welches jetzt frei von Colchicin ist, fängt an, zum normalen Zustand zurückzukehren. Dabei erholen sich die Ruhezellen bereits zu normalem Zustande.

17.24. Die Chromosomen sehen blaß und ein wenig unklar aus.

17.55. Der Erholung der Spindeltätigkeit entsprechend, tritt nach und nach die Polarität zwischen den zerstreuten Chromosomen ein, wobei die Differenzierung der Spindelfasern auch im Atraktoplasma vermutet wird (Fig. 4). Das Atraktosom erholt sich zu seiner Spindelform und die Chromonematisierung einzelner Chromosomen beginnt.

18.07. Drei Stunden nach der Behandlung. Infolge des Zerstreuens der Halbchromosomen vereinigen sie sich nicht mehr zu einem Chromosomenklumpen, sondern zu drei Gruppen, wo sie zusammenschrumpfen und sehen jetzt kurz und dick aus. Zwischen diesen Chromosomengruppen häuft sich das Atraktoplasma als phragmoplast-bildende Substanz an und bildet zwei hyaline Räume. Später entwickeln sich diese Räume, jeder für sich, zu einem Phragmoplast (Fig. 5 *x*), in welchem eine Wandanlage erscheint (Fig. 5 *y*). Um den Phragmoplast herum sammeln sich granulenreiche flüssige Zytoplasma an.

18.24. Schließlich wird die Mutterzelle mit zwei Scheidewänden in drei Tochterkerne und drei Tochterzellen geteilt.

2. August. Ein Tag nach der Behandlung. Die Struktur der Tochterkerne ist normal und jede Tochterzelle weist lebhafte Zytoplasmaströmungen auf (Fig. 6).

4. August. Die Tochterzelle, die sich an der Spitze der Mutterzelle befindet, stirbt an Koakulation ab, aber die anderen sind in ihren Strukturen normal und gesund.

Aus dem Verhalten der Teilungsfigur bei dieser Colchicin-Behandlung werden die Veränderungen des Atraktoplasmas nicht als chemisch sondern als physikalisch angesehen und zwar gehen sie reversibel vor sich. Es kommen jedoch verschiedene Teilungsanomalien als Nachwirkung der Colchicin-Behandlung zustande. Die Umkehrbarkeit der Spindeltätigkeit bei der C-Mitose wurde bereits von LEVAN (1938) bei den Zellen der *Allium*-Keimwurzel festgestellt. Bei seinen Versuchen setzte die Regeneration der Spindelfigur nach

Verlauf von 12–24 stündigem Verweilen im Wasser ein und die Mitose ging in 36–48 Stunden in normaler Weise vor sich. In der Übergangszeit von den typischen C-Mitosen zu normalen Mitosen erschienen verschiedene Teilungsanomalien, nämlich multipolare, asymmetrische oder nicht verdichtete Spindeln usw. Eine ähnliche Nachwirkung bei der C-Behandlung bemerkte SATÔ (1939) bei Pollenmutterzellen der Aloinae-Arten.

Die Ursache dieser Mannigfaltigkeit der Teilungsfiguren bei der Wiederherstellung der Spindeltätigkeit beruht nicht nur auf unvollkommene Tätigkeit der Spindelfigur sondern auch auf der zufälligen Verteilung der Teilungselemente in der Zelle, nämlich auf dem Zerstreuen der Chromosomen, der Anhäufungsstelle des Atraktoplasmas und weiter auf der Verteilung der Zytoplasmamasse und der Vakuolen im Zellraum.

2. Versuche mit Medien verschiedener Konzentrationen: Um die Konzentration der Colchicinlösung, deren Einwirkung auf die Mitose man durch Lebendbeobachtung sofort entscheiden kann, festzustellen, wandte ich die Methode 2 an. Im Medium von 0,005-proz. Colchicinlösung ist seine Wirkung auf die Mitose nach Verlauf von mehr als zweistündigen Verweilen in der Lösung zu erkennen. Daher geht die Mitose dabei normal vor sich, bevor das in die Zelle eingedrungenen Colchicin auf die Mitose einwirkt, da die intakte Teilungszelle bei *Tradescantia*-Haarzellen ihre Mitose in weniger als zwei Stunden vollendet.

Im Medium einer 0,01-proz. Colchicinlösung wirkt das Colchicin auf das Atraktoplasma langsam und nur schwach ein; in der Anaphase können die Halbchromosomen zum Spindelpol wandern, aber sich dort kaum zusammenballen. Sie gestalten sich in der Telophase zuerst zu einem erweiterten, aber später zu einem dichten Tochterkerne. Wie bei anderen Experimenten sistiert die Entwicklung der Chromatinfäden zu Chromosomen bei den isolierten *Tradescantia*-Haarzellen oft durch Eindringen des Colchicins, und die Rückbildung des Prophasekernes zum Ruhekern erfolgt (Fig. 44–45).

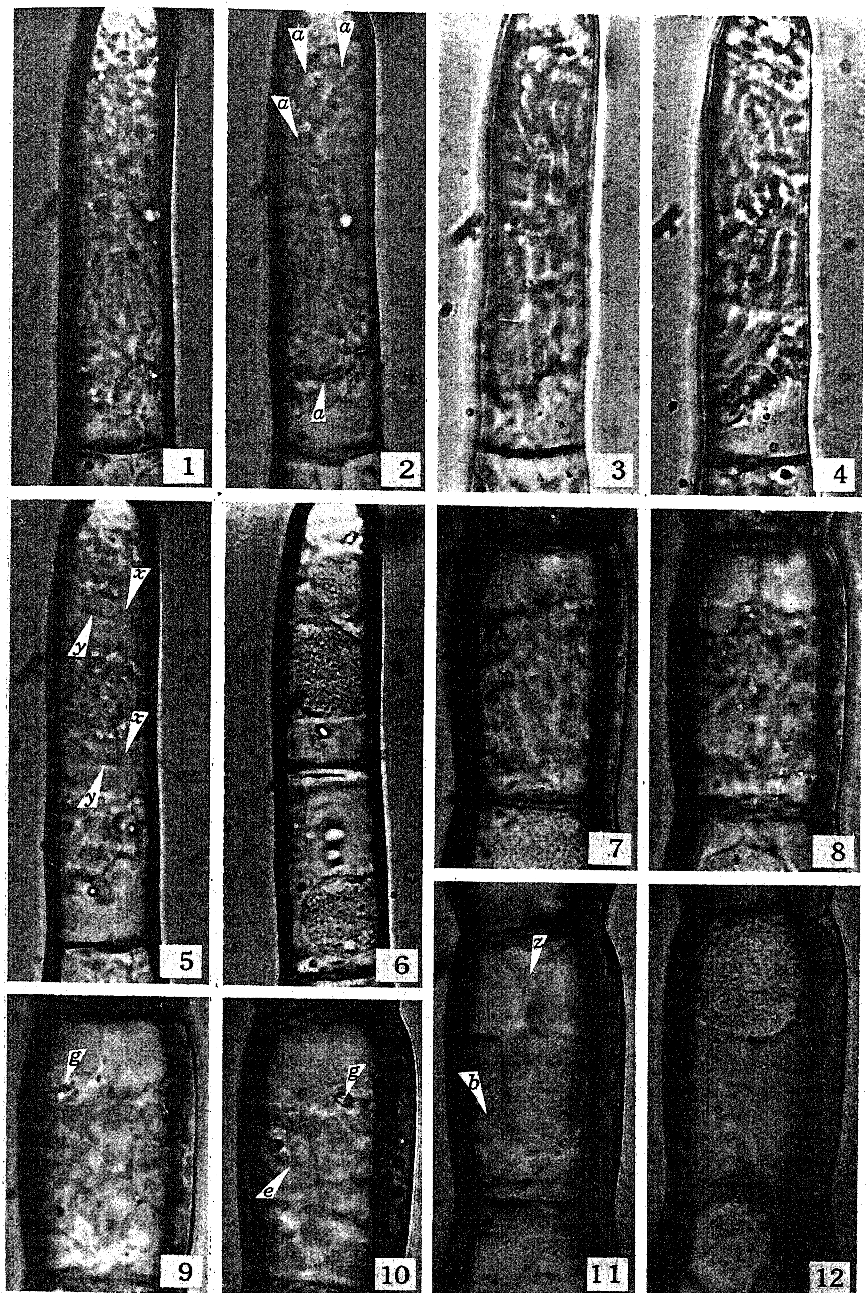
Im Medium einer 0,05-proz. Colchicinlösung tritt die Veränderung der Mitose in einigen Minuten auf. In der Metakinese oder Metaphase verliert das Atraktoplasma seine Tätigkeit, bevor die voneinander getrennten Halbchromosomen die Spindelpole erreichen. Daraus erklärt sich das Unterbleiben der Chromosomenwanderung zum Spindelpol; es entstehen dabei weder der Phragmoplast noch die Wandanlage, und die Halbchromosomen bilden einen Didiploidkern. Dagegen erreichen die Halbchromosomen bei der mittleren oder späteren Anaphase die Spindelpole und vollenden die Kernteilung, da

die Anhäufung des Colchicins in der Zelle nicht rechtzeitig erscheint, um die Chromosomenwanderung zum Spindelpol zu hindern und zwar weil die Wanderung der Chromosomen im Vergleich mit den vorderen Fällen in kurzer Zeit zu Ende ist. Aber die Anhäufung des Colchicins in der Zelle erscheint zeitig genug, um die Entwicklung der Wandanlage im Phragmoplasten zurückzuhalten, wodurch eine zweikernige Zelle entsteht (Fig. 31–34).

Im Medium einer 0,1-proz. Colchicinlösung wird die Tätigkeit der sich teilenden Zellen sofort gestört; diese Konzentration eignet sich für den Zweck, die Colchicin-Wirkung auf ein beliebiges Teilungsstadium durch Lebendbeobachtung zu verfolgen. Die 0,1-proz. Lösung stört die Chromosomenbildung im Prophasekern nicht immer, dagegen wird das Atraktoplasma unter dieser Konzentration immer beeinflußt, und dadurch entstehen verschiedene C-Mitosen.

Im Medium einer 0,5-proz. Colchicinlösung sistiert die Tätigkeit des Atraktosoms sofort; die Lösung wirkt sowohl auf die Teilungsfigur als auch auf das Zytoplasma allmählich schädlich, indem Gelifikation der Teilungselemente eintritt und die Lebenstätigkeit der Zellen geschwächt wird. Im Gegensatz zum Medium einer 0,5-proz. Lösung hat dasselbe einer 0,1-proz. auf die Lebenstätigkeit der Zellen kaum eine schädliche Wirkung. In der Tat haben die Zellen in der 0,1-proz. Colchicinlösung eine Lebensfähigkeit von einigen Tagen bis zu einigen Wochen und können die Protoplasmaströmungen fortsetzen. Die hier angegebenen Konzentrationen des Colchicins, die sofort auf die Teilungsfigur einwirken, weichen von der niedrigsten Konzentration der Lösung ab, deren Einwirkung auf die Mitose erst in einigen Stunden nach der Behandlung auftritt. Nach Versuchen von NEBEL und RUTTLE (1938) bei der Mitose von *Tradescantia*-Haarzellen erweist sich die 0,004-proz. Colchicinlösung als die niedrigste Konzentration; nach LEVAN (1938) wurden bei Mitosen der *Allium*-Keimwurzel 0,005–0,01-proz. Lösungen als Minimalkonzentration betrachtet. Versuche von WALKER (1938a), bei welchen Blütenstengel von *Tradescantia paludosa* in einer 0,1-proz. Colchicinlösung 24–66 Stunden lang stehen gelassen wurden, wiesen eine Vermehrung der Chromosomenzahl im Gewebe der Fruchtknoten erst nach 5 Tagen und bei Staubfadenhaarzellen etwas früher auf.

Fig. 1–6. Veränderung der Spindelfigur und ihre Umkehrbarkeit. Erweitertes Atraktosom, C-Paarungen der Chromosomen und eine abnorme Scheidewandbildung. Eingehende Erklärung im Text (siehe S. 95). **Fig. 7–12.** Chromosomenverdoppelung in einer 0.05-proz. Lösung. 7. 4. Aug. 1939. 10.50. Metakinese. 4 Minuten nach der Behandlung. 8. 11.07. Erweiterter Zustand des Atraktoplasmas. 9. 11 22. Einsetzen der Veränderung der Chromosomenmatrix. 10. 11.54. Gepaarte Chromosomenden sichtbar. 11. 13.06. Anhäufung beweglicher flüssiger Plasma. 12. 5. Aug. *a*



Grenzfläche des erweiterten Atraktoplasmas, x Phragmoplast, y Wandanlage, k Einbuchtung an der Kernoberfläche, b flüssige Plasmamasse. Durch lebhafte Bewegung der Granulen im Plasma oder durch Strömung des Plasmas selbst ist die Anhäufung des flüssigen Plasmas in der Photo (wie auch in den folgenden Textfiguren) nicht deutlich erkennbar.

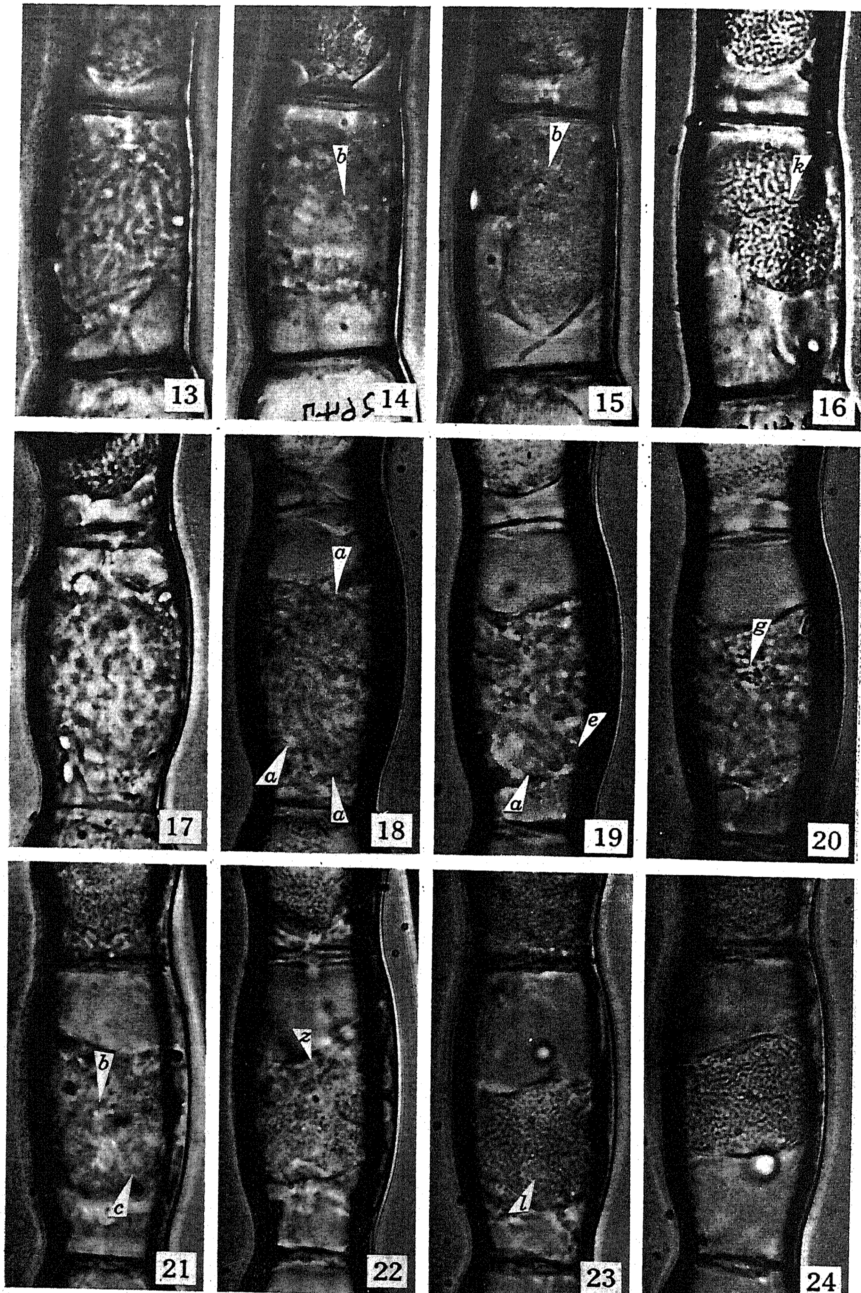


Fig. 13–16. Anhäufung des flüssigen Plasmas beim Abbau des Atraktosoms in einer 0.1-proz. Lösung. 13. 4. Aug. 1939. 8.58. Mittlere Anaphase. 5 Minuten nach der Behandlung. 14. 9.13. Anhäufung des granulenreichen Plasmas. 15. 9.38. Verflüssigung der Plasmaanhäufung. 16. 5. Aug. Fig. 17–24. Chromosomenverdopplung bei einem Prophasekern. Eingehende Erklärung im Text (siehe S. 105). *a* Grenzfläche des Atraktosoms, *e* Chromosomende, *c* sich chromonematisierende Chromosomen, *g* granulenreiches Plasma, *b* Anhäufung des flüssigen Plasmas, *z* Plasmaströmungen, *l* Nukleolus.

3. **Atraktoplasma:** Das Eindringen des Colchicins in die Zelle ruft zuerst eine Aktivität des Zytoplasmas am Wandbelage und um das Atraktosom hervor. Unter den Granulen im Zytoplasma setzt in einer 0,1-proz. Lösung gleich eine lebhafte Bewegung ein, jedoch finden Zytoplasmaströmungen um das Atraktosom nicht sofort statt. In Ruhezellen tritt aber nicht nur eine lebhafte Bewegung der Granulen im Zytoplasma, sondern auch die des Zytoplasmas auf. Hier muß ich jedoch besonders bemerken, daß diese Beschleunigung der Granulenbewegung im Zytoplasma, nämlich die Abnahme der Zytoplasmaviskosität unter der Colchicinlösung keineswegs für die direkte Ursache der C-Mitose gehalten werden soll. Die unmittelbare Veranlassung der C-Mitose hängt vor allem von der Veränderung des Atraktoplasmas ab.

Wie im normalen Zustand unterscheidet sich das Atraktoplasma auch unter der Colchicinlösung durch eine Grenzfläche vom Zytoplasma; es ist ohne Vermischung mit dem Zytoplasma ständig um die Chromosomen vorhanden, ist noch granulenfrei und sieht blässer als das Zytoplasma aus (Fig. 2, 8, 37). Aber das Atraktosom sieht beträchtlich erweitert aus, und seine Grenzfläche wird uneben und ist schwer festzustellen. Im Innern des erweiterten Atraktosoms ordnen sich die Chromosomen so zerstreuend, daß es ihnen nunmehr unmöglich ist, eine Äquatorialplatte zu bilden, zu Spindelpolen zu wandern und sich dort zu Tochterkernen zusammmenzuballen. Aus diesem Verhalten der Chromosomen und des Atraktoplasmas ergibt sich, daß ein neuer Gleichgewichtszustand zwischen den Teilungselementen hervorgebracht wird, zu dem eine Abnahme der Oberflächenspannung des Atraktoplasmas, wie auch eine Abnahme einer Außenkraft auf die einzelnen Chromosomen angenommen werden (Fig. 48 a, b).

Die C-Paarung in lebenden Zellen ersieht man in Fig. 3, 39 und die gepaarten Chromosomenden in Fig. 10. Jedoch stößt man auf Schwierigkeiten, die Centromeren der gepaarten Chromosomen bei Lebendbeobachtung zu finden. Bei *Tradescantia*-Haarzellen treten die C-Paarungen bei 0.1-proz. Lösung manchmal, aber bei der Methode 3 fast immer auf, da das Colchicin bei der Methode 3 langsamer in die Zelle eindringt als bei einer 0,1-proz. Lösung. LEVAN (1938) bemerkte, daß die C-Paarung am häufigsten einige Stunden nach der Behandlung in Erscheinung tritt. Auch SATÔ (1939) berichtete, daß die Univalenten bei der Reduktionsteilung durch lange Behandlung viel häufiger entstehen.

In konzentrierten Colchicinlösungen gehen die Veränderung des Atraktoplasmas und der Chromosomen schnell vorüber; infolge der sofortigen Chromonematisierung und der Zytoplasmatisierung endet

nämlich die Verdoppelung der Chromosomenzahl auch schnell, ohne daß sich die Chromosomen weit zerstreuen und ohne viele C-Paarungen zu bilden. Die Ergebnisse bei den Versuchen verschiedener Colchicin-Konzentrationen beweisen, daß bei den verschiedenen Pflanzen und deren einzelnen Geweben, eine jeweils günstigste Konzentration und Behandlungszeitdauer für die Chromosomenverdoppelung und auch für die C-Paarung vorhanden sind. Jedoch erweist sich die C-Paarung selbst durchaus nicht als ein unentbehrlicher Vorgang für die Chromosomenverdoppelung.

Obwohl viele Forscher bei fixierten Präparaten die Exsistenz der Spindelsubstanz in der C-Mitose übersehen haben, bedeutet die Untätigkeit der Spindelfigur keineswegs das Nicht-Vorhandensein der Spindelsubstanz, wie ich ihre Exsistenz bei der C-Mitose in lebenden Zellen festgestellt habe. Hier wende ich mich zum Schicksal des Atraktoplasmas bei der C-Mitose. Fast gleichzeitig mit der Chromonematisierung der Chromosomen setzt der Abbau des Atraktoplasmas ein. Dem Zerfall des Atraktoplasmas vorangehend, treten Granulen an der Oberfläche des Atraktosoms auf, einige davon bilden eine Gruppe und setzen die Brownsche Bewegung fort (Fig. 9, 10, 20).

Über den Ursprung dieser Granulen kann ich zwei Erklärungen anführen; diese Granulen sind teils auf Granulen im Zytoplasma, welches das Atraktosom deckt, und teils auf Fällungen beim Abbau des Atraktoplasmas zurückzuführen, die sich durch Colchicin im Atraktosom gestalten, obwohl man oft nicht entscheiden kann, welche Granulen zur ersten Art und welche zur zweiten gehören. Während der Colchicin-Behandlung bemerkte ich jedoch einige Mal Fällungen im Atraktoplasma, welche im erweiterten Atraktosom als winzige Granulen auftreten.

Beim Zerfall des Atraktoplasmas ist die Zunahme der Fluidität des Atraktoplasmas auffallend; zugleich setzt die Strömung des Atraktoplasmas ein und die Granulen an seiner Oberfläche bewegen sich. Während der Verflüssigung des Atraktoplasmas sehen die Chromosomen vorübergehend unklar aus. Das bedeutet, daß eine Veränderung der Chromosomenmatrix bereits stattfindet (Fig. 14, 27). Aber wenn die Gestalt der Chromosomen wieder erkennbar wird, ersieht man das Einsetzen ihrer Chromonematisierung und das Einsetzen der Strömungen des verflüssigten Atraktoplasmas zwischen ihnen. Das stark verflüssigte Atraktoplasma häuft sich neben der Chromosomenmasse an, und bildet dort eine granulenreiche flüssige Plasmamasse, wobei sich die Chromonematisierung der Chromosomen ihrem Ende nähert (Fig. 11, 15, 29). Derselbe Zusammenhang zwischen der Chromonematisierung der Chromosomen und der

Zytoplasmatisierung des Atraktoplasmas wurde auch schon bei den Versuchen mit dem Dampfgemisch Ammonia-Chloroform festgestellt (WADA 1939, S. 177). Bei den Colchicin-Versuchen nehme ich auch an, daß sich das veränderte Atraktoplasma beim sofortigen Verschwinden des Colchicins in der Teilungszelle zur phragmoplastbildenden Substanz verändern kann, jedoch bleibt unter der dauernden Wirkung des Colchicins das veränderte Atraktoplasma als Phragmoplast-Substanz nicht zurück; es verändert sich weiter ohne Bildung des Phragmoplasten zur Zytoplasmanatur. Was die Zytoplasmatisierung des Atraktoplasmas anbetrifft, so handelt es sich um den endgültigen Zustand des abgebauteten Atraktoplasmas. Beim Verschwinden des Atraktoplasmas im Zytoplasma wissen wir heute noch nicht, ob das Atraktoplasma dabei chemisch mit dem Zytoplasma übereinstimmt, oder ohne Vermischung mit dem Zytoplasma in ihm submikroskopisch eine Dispersionsphase bildet. Auf alle Fälle ist es unmöglich das abgebautete Atraktoplasma vom Zytoplasma morphologisch zu unterscheiden. Diese Veränderungen des Atraktoplasmas bezeichne ich als Zytoplasmatisierung (Fig. 48 c, d).

Die Chromonematisierung einzelner Chromosomen und die Zytoplasmatisierung des Atraktoplasmas unter der Colchicin-Wirkung schreiten oft von einer Seite der Teilungsfigur aus zu einer anderen fort (Fig. 28 *c*). Bei *Allium*-Zellen beweist LEVAN (1938) den Nicht-Synchronismus der Colchicin-Wirkung, da die Teilung der Centromeren in einem Chromosomensatz nicht simultan stattfindet. Leider war ich nicht imstande durch Lebendbeobachtung die Teilung der Centromeren bei der Mitose der *Tradescantia*-Haarzellen zu verfolgen.

Ich überzeugte mich davon, daß sich die Spindelfasern, die in normaler Mitose im Atraktoplasma differenzieren, unter der Colchicin-Wirkung nicht mehr gestalten und daß sich solche, die sich vor der Behandlung bereits im Atraktoplasma befinden, entdifferenzieren und später zytoplasmatisieren. WALKER (1938) nahm an, daß die Entstehung der C-Mitose auf der Zurückhaltung der Spindelfaserbildung beruht. Aber die Lebendbeobachtung verschiedener Verhalten der C-Mitose beweist, daß bei der Entstehung der C-Mitose die qualitative Veränderung des Atraktoplasmas die Hauptrolle spielt, während die Zurückhaltung der Spindelfasern nur eine Nebendeutung hat.

4. Prophase: Daß die Entstehung der C-Mitose auf der Störung des Spindelmechanismus beruht, ist jetzt von allen Forschern anerkannt. Aber hinsichtlich des Verhaltens des Prophasekernes muß festgestellt werden, ob die Chromosomenverdoppelung ohne Eintritt in die Metaphase möglich ist oder nicht. Unter der Colchicin-Wir-

kung entwickeln sich die Chromatinfäden bei früheren Prophasekernen isolierter *Tradescantia*-Haarzellen zu Chromosomen (Fig. 17–24, 25–30), obwohl es dabei auch oft vorkommt, daß die Entwicklung der Chromatinfäden zu Chromosomen zurückgehalten wird und daß die Prophasekerne dann zur Ruhekernstruktur zurückkehren (Fig. 44–47).

Wie ich schon bei Anstichversuchen und auch bei anderen Experimenten festgestellt habe, stehen die Entwicklung der Chromatinfäden zu Chromosomen und dieselbe der Karyolymphe zum Atraktoplasma im Prophasekern im Zusammenhang (WADA 1935). Weiter habe ich auch auf die Möglichkeit hingewiesen, daß die Chromosomen, das Atraktoplasma und die noch nicht zum Atraktoplasma differenzierte Karyolymphe zusammen im späten Prophasekern vorhanden sind. Wird ein solcher später Prophasekern mit einer Colchicinlösung behandelt, so verändert sich das Atraktoplasma leicht, die anderen Kernelemente jedoch bleiben fast unverändert. Daher geht die Entwicklung der Schwesterchromatiden zu Chromosomen in diesem veränderten Atraktoplasma ohne Hindernis vor sich und die Chromosomenzahl wird dadurch ohne Bildung eines geformten Spindelapparates verdoppelt (Fig. 18–19, 25–26).

Der deformierte Kernraum in der späten Prophase ist nicht so groß wie der deformierte Spindelraum in der Metaphase. Daher ist der Kernraum von Halbchromosomen dicht gefüllt; hier und da ersieht man Gruppen von zwei oder vier Chromosomenden (Fig. 19). Weitere Veränderungen des Kerninhaltes gehen in derselben Weise vor sich, wie die in einer behandelten Spindelfigur; die Chromosomen sehen nämlich zuerst durch Abbau der Chromosomenmatrix unklar aus, dann prägt sich die Chromonematisierung einzelner Chromosomen aus. Während dieser Chromonematisierung schreitet die Zytoplasmatisierung des Atraktoplasmas auch fort und das Atraktoplasma verändert sich schließlich zu einem flüssigen granulenreichen Plasma (Fig. 21, 29).

Aus diesen Versuchen ergibt sich, daß die Verdoppelung der Chromosomenzahl bei dem Prophasekern durch Colchicin-Behandlung auch möglich ist, ohne daß der Kern in die Metaphase eintritt und ohne die Chromosomen in die Kernplatte anzuordnen (Fig. 24, 30). Die Verdoppelung der Chromosomenzahl bei dem Restitutionskern in Fig. 24 und 30 wird durch Aufzählen der Chromosomenzahl bei der nächsten Kernteilung festgestellt werden. Jedoch kann man aus der Gestalt dieser Kerne auf die Chromosomenverdoppelung schließen, da die gespaltenen Halbchromosomen, die zu einem Riesenkern verschmelzen, oft die Neigung zeigen, die homologen Chromosomen von einander getrennt in zwei Massen zu vereinigen und sich dadurch

zu einer Kokonform mit einer schwachen Einbuchtung an der Mitte der Kernoberfläche zu gestalten (vgl. WADA 1935, S. 388).

Über die Colchicin-Wirkung auf die Prophasekerne gebe ich hier ein Beispiel (Fig. 17–24). Die behandelte Teilungsfigur befand sich in der dritten Zelle des Haarendes von *Tradescantia reflexa*. Als Untersuchungsmedium benutzte ich eine 0,1-proz. Colchicinlösung, die mit einer 2-proz. Rohrzuckerlösung verdünnt war.

10.34 am. 14. August 1939. Ein isoliertes, einen mittleren Prophasekern enthaltendes Haar wird ins Medium eingetaucht.

10.36. Der Kern ist grobknäuelförmig und seine Peripherie ist teilweise unsichtbar (Fig. 17).

10.41. Das Zytoplasma entmischt sich. Die Chromatiden zeigen sich einzeln klarer, und liegen nebeneinander, von einander gelöst. Im Kernraum ist die Entwicklung des Atraktoplasmas aus der Karyolymphe erkennbar (Fig. 18).

10.53. Durch die weitere Entwicklung einzelner Chromatiden zu selbstständigen Chromosomen, wird die Verdoppelung der Chromosomenzahl im Kernraum durchgeführt. Jedoch bemerkt man weder die Spindelfigur noch die Kernplatte in der Zelle. Das Atraktoplasma, welches die Chromosomen einhüllt, sieht blaß aus und unterscheidet sich mittelst einer Grenzfläche und auch durch Fehlen der Granulen vom Zytoplasma.

11.21. An der Oberfläche des Atraktosoms treten winzige Granulen auf, sie treffen zu je einigen zu Gruppen zusammen und setzen die BROWNsche Bewegung fort. Im Atraktosom treten hier und da die gepaarten Enden der Schwesterchromosomen auf (Fig. 19).

11.35. Der Kerninhalt bleibt fast unverändert.

12.16. Das Aussehen der Chromosomen wird etwas unklar. Bewegung der Granulen an der Oberfläche des Atraktosoms setzt ein (Fig. 20).

13.20. Das Medium ist durch ein frisches ausgetauscht.

13.29. Im Atraktosom schreitet einerseits die Chromonematisierung der Chromosomen rasch fort; andererseits verändert sich das Atraktoplasma zu flüssigem Zytoplasma. Dieses flüssige Zytoplasma bewegt sich zusammen mit den Granulen zu dem einen Rande der Chromosomenmasse (links oben in Fig. 21 *b*), und bildet dort eine granulenreiche flüssige Zytoplasmamasse. Im unteren Teil der Fig. 21 machen sich noch einige Chromosomen, deren Spiralstruktur noch etwas erkenntlich ist bemerkbar (Fig. 21 *c*).

13.53. Die Chromosomen vollenden die Chromonematisierung und entwickeln sich zu einem Ruhekern. Von der flüssigen Plasmamasse dehnen sich einige Zytoplasmastränge aus und zeigen lebhafte Plasmaströmungen (Fig. 22 *z*).

15.21 Der Kern quillt noch etwas an und ein großer Nukleolus ist erkennbar (Fig. 23 *l*).

15. August. Die Struktur des Kernes ist ganz normal und in der Zelle zeigen sich lebhafte Zytoplasmaströmungen (Fig. 24).

22. August. Die Zelle stirbt an Koagulation ab.

5. **Phragmoplast:** Hier wende ich mich zur Colchicin-Wirkung auf den Phragmoplast. Durch Eindringen des Colchicins in die telophasische Zelle treten winzige Granulen sofort im Phragmoplast auf, der vor der Behandlung ein hyaliner Raum und frei von Granulen war (Fig. 31). Der mit der Methode 1 behandelte späte anaphasische Kern in Fig. 31 zeigt den Colchicin-Effekt erst in der Telophase. Die Granulen im Phragmoplast nehmen von der Peripherie nach dem Innern an Zahl zu und ihre lebhafte Bewegung beweist die Zunahme

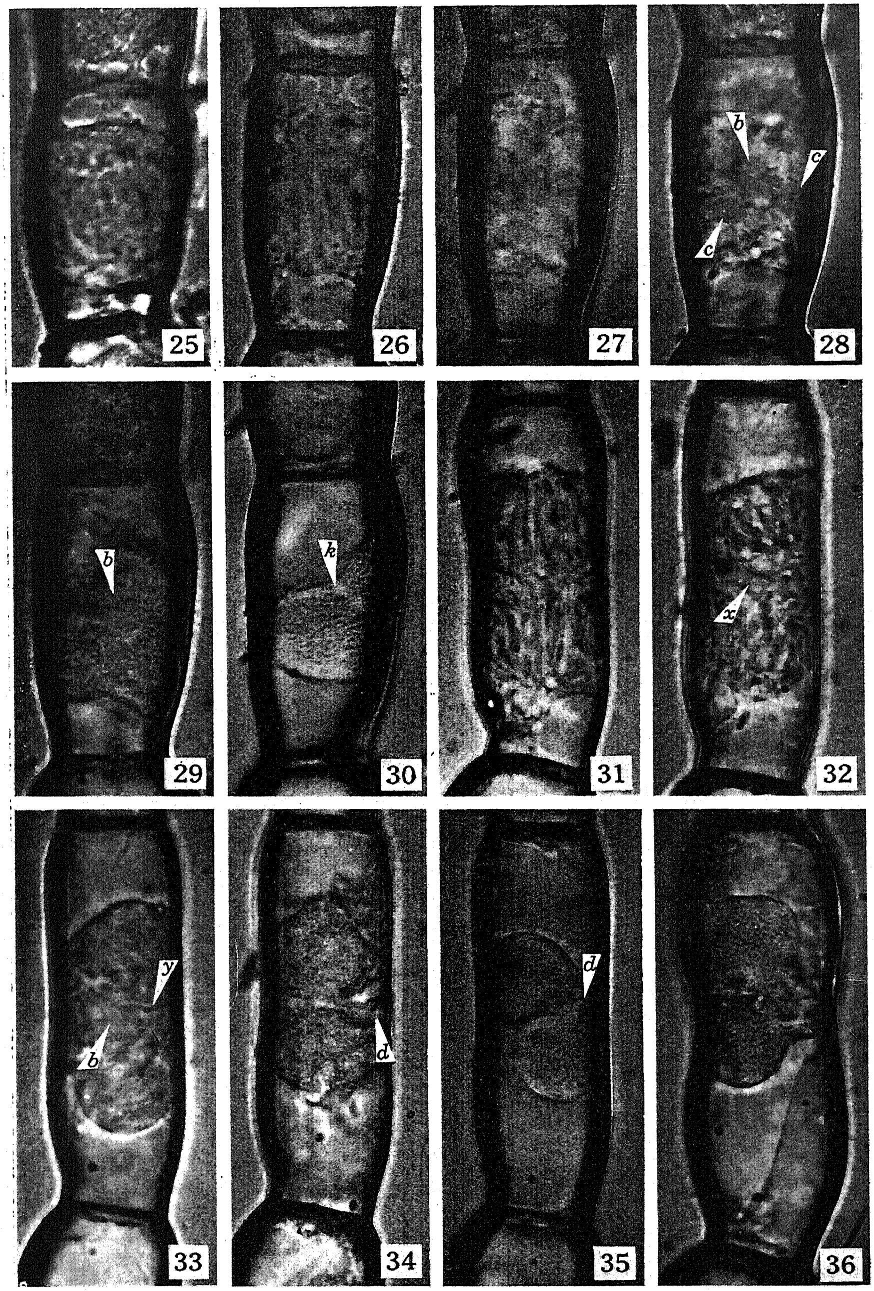
der Fluidität der Phragmoplast-Substanz. Durch diese Vorgänge verändert sich die Phragmoplast-Substanz zur Zytoplasmanatur. Dann entsteht an der Stelle des Phragmoplasten eine flüssige granulenreiche Plasmamasse, die später mit dem Zytoplasma der Mutterzelle verschmilzt und zusammenfließt.

Wenn die Wandanlage sehr jung ist, verschwindet sie durch Colchicin-Behandlung während der Zytoplasmatisierung der Phragmoplast-Substanz im Zytoplasma und es entsteht eine zweikernige Zelle. Wenn sich die Wandanlage schon zu einer Zellplatte entwickelt hat, oder an einer Seite derselben die Mutterzellwand erreicht hat, entwickelt sie sich im ersteren Fall zur Scheidewand, aber im letzteren nur zu einem Wändchen, welches sich als Faltung im Innern der Mutterzellwand hervorhebt (Fig. 33 *y*, 34 *d*, 35 *d*).

Diese Ergebnisse zeigen, daß das Colchicin die Entstehung einer neuen Wandanlage und die Entwicklung einer jungen Wandanlage zur fertigen Scheidewand verhindert. Aber die Zellplatte leidet unter der Colchicin-Wirkung kaum und sie entwickelt sich zur Scheidewand. Daher entstehen durch Colchicin-Behandlung eine zweikernige Zelle oder eine solche mit einem Wandreste aus einer früheren Telophase und eine fertige Scheidewand aus einer mittleren oder späteren Telophase. Die Tatsache, daß die Zytoplasmatisierung des Atraktoplasmas und diejenige der Phragmoplast-Substanz im Wesen nicht unterschieden werden können, beweist daß die beiden Teilungselemente stofflich in inniger Beziehung miteinander stehen, obwohl sie morphologisch besonders in fixierten Präparaten verschieden aussehen.

6. Einige anderen Kernanomalien bei der C-Mitose: Unter der Colchicin-Wirkung verteilen sich die Chromosomen im Atraktoplasma so sporadisch, daß die Rekonstruktion des Ruhekernes oft Gefahr läuft, aus den Chromosomen von Anfang an einen kugeligen Riesenkern zu bilden. Die Chromosomen bilden daher unförmliche Ruhekerne oder solche mit einigen Auswüchsen (Fig. 40, 41). Aber die erweiterte Struktur der Ruhekerne verdichtet sich allmählich und erkleinert sein Volumen, wobei kleine eingeschnürte Auswüchse sich oft vom Ganzen trennen und Sonderkerne bilden. Die Restitutionskerne zeigen sich also schließlich als eine massive Kugelform, oder sehr oft als solche mit ein oder zwei Einbuchtungen an der Oberfläche (Fig. 16, 43, 46). Es ist auch bemerkenswert, daß sich erweiterte

Fig. 25–30. Chromosomenverdoppelung beim Prophasekern in einer 0.1-proz. Lösung. **25.** 8. Aug. 1939. 15.18. Stadium der groben Knäuel. 8 Minuten nach der Behandlung. **26.** 15.33. Erweiterte Chromosomen. **27.** 17.03. Unklarwerden der Chromosomen. **28.** 17.34. Abbau des Atraktoplasmas und Chromonematisierung der Chromosomen. **29.** 17.47. Anhäufung des flüssigen Plasmas. **30.** 9. Aug. **Fig. 31–36.** Veränderung des Phragmoplasten, behandelt nach Methode 1. **31.** 1. Aug. 1939. 10.04. Späte



Anaphase. Vor der Behandlung. **32.** 10.11. 5 Minuten nach der Behandlung. Granulierung der Phragmoplast-Substanz. **33.** 10.20. Eine Wandanlage sichtbar. **34.** 11.17. Ein Wandrest. **35.** 2. Aug. Zweikernige Zelle mit einem Wandrest **36.** 4. Aug. Die Zelle wächst beträchtlich. *b* flüssiges Plasma, *y* Wandanlage, *d* Wandrest, *x* Phragmoplast.

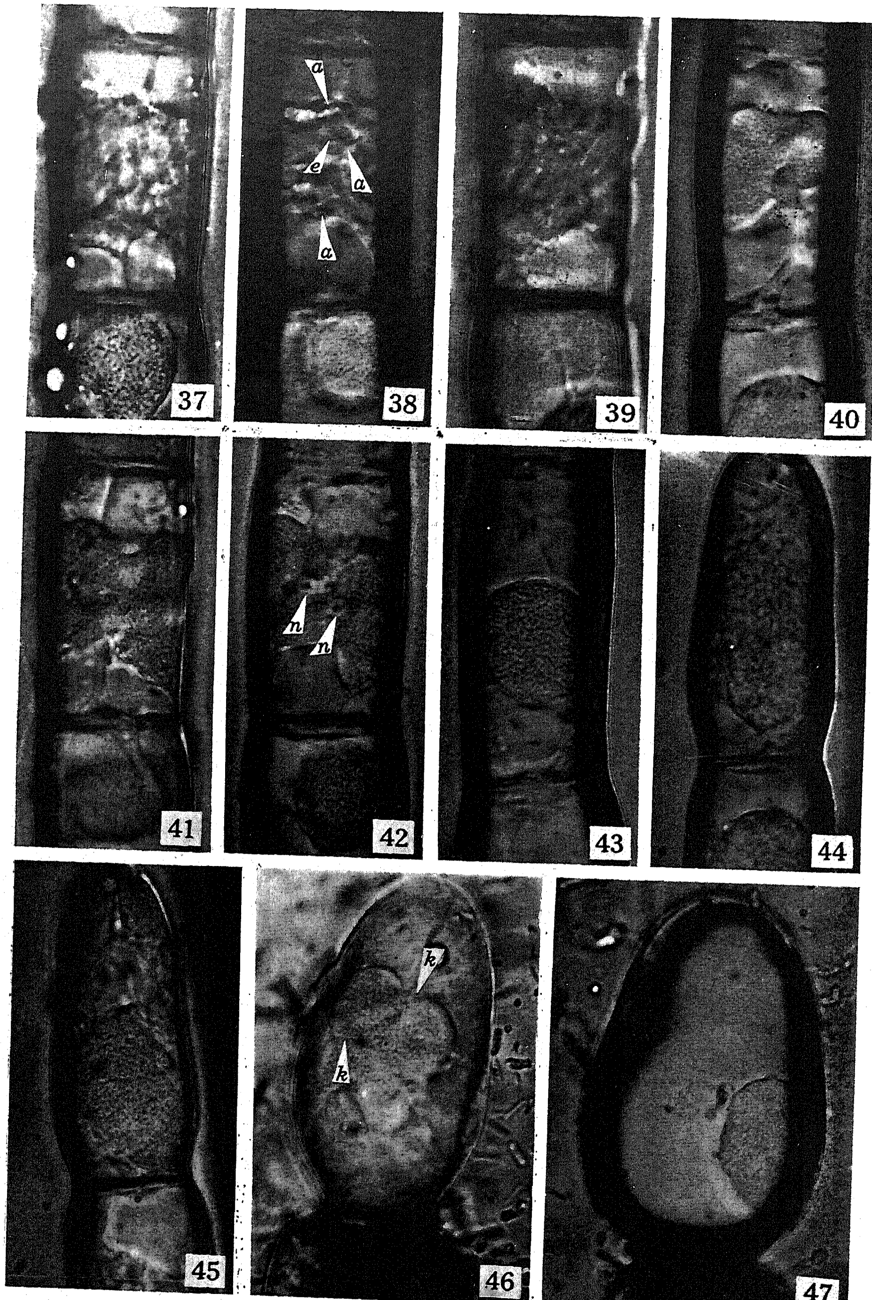


Fig. 37–43. Chromosomenverdoppelung durch die Methode 3. Eingehende Erklärung im Text (siehe S. 109ff). Fig. 44–47. Teilungsumkehr bei einem frühen Prophasekern und Kultur in einer 0.1-proz. Lösung. 44. 9. Aug. 1939. 8.46. Frühe Prophase. 6 Minuten nach der Behandlung. 45. 10.16. Zurückgekehrter Ruhezustand des Kernes. 46. 22. Aug. Veränderung der Kerngestalt während der Kultur. 47. 21. Sept. Zellwachstum. *a* Grenzfläche des erweiterten Atraktosoms, *n* Vakuolen im Kern, *k* Einbuchtung an der Kernoberfläche.

Riesenkerne erst nach 2–3 tägigem Zeitverlaufe zur verdichteten Kugelform verwandeln.

Im erweiterten Restitutionskern werden oft Zytoplasmateile eingeschlossen. Solche eingeschlossene Substanzen pflegen sich später zu verflüssigen und bilden Vakuolen im Kern (Fig. 42). Diese Kernvakuolen werden aber im Verlaufe der Zeit vom Kern ausgeschieden, wie ich dasselbe Verhalten der Kernvakuolen bei den Anstichversuchen schon beschrieben habe (WADA 1934). Im Vergleich mit den Ergebnissen dieser Lebendbeobachtungen scheinen mir das Zustandekommen unförmlicher Kerne und das der Kerne mit einigen Löchern oder mit einigen Kanälen bei den LEVANschen Versuchen auch auf einem zufälligen Eintritt der Zytoplasmateile in den erweiterten Kern und auf der Verflüssigung dieser zytoplasmatischen Beisätze zu beruhen, und diese verschiedenen Gestalten der Riesenkerne werden nicht immer als die endgültige Form, sondern als eine Übergangsform der Restitutionskerne angenommen, die sich von einer erweiterten Struktur zu einer verdichteten verwandelt.

Bei der schon mit Erfolg angewandten Colchicin-Behandlung, bei welcher die meristematischen Gewebe für eine lange Zeitdauer in verdünnter Colchicinlösung verbleiben, beobachtete ich den Verlauf der C-Mitose nicht in fixierten sondern in lebenden Zellen und gebe hier ein Beispiel zu Protokoll. Durch dieselbe Colchicin-Behandlung untersuchten NEBEL und RUTTLE (1938) auch die Mitose der *Tradescantia*-Haarzellen, wobei sie die Zellen bei der Beobachtung mit Azetokarmin abtöteten.

Für die Lebendbeobachtung isolierte ich ein Staubfadenhaar von *Tradescantia reflexa* aus einer mit der Methode 3 behandelten Blütenknospe, und brachte es in die Feuchtkammer, wobei ich eine 2-proz. Rohrzuckerlösung als Medium benutzte.

11.30 am 21. Juli 1938. Einige junge Blütenknospen werden in eine 0,004-proz. Colchicinlösung eingetaucht.

13.20. Ein Staubfadenhaar wird von einer der Knospen isoliert und in die Feuchtkammer eingebracht, wo sich eine Mitose in der dritten Zelle vom Haarende aus befindet.

13.24. In der Colchicinlösung erreicht die Teilungsfigur schon die Metakinese, bevor das Colchicin in der Zelle eine Wirkung ausübt, da sich sowohl die Polplasmastränge als auch das Atraktoplasma ziemlich gut entwickeln. Die Veränderung der Teilungsfigur geht dabei nur langsam vor sich (Fig. 37).

14.18. Die Chromosomen quellen an und die homologen liegen parallel, aber losgelöst voneinander. In Fig. 38 sieht man nur den oberen Teil des Atraktosoms, in welchem einige Chromosomenden Paare bilden. Die Stelle, wo die Chromosomenden liegen, ist frei von Granulen und durch eine Grenzfläche vom Zytoplasma unterschieden, obwohl die Gestalt des Atraktosoms hier merklich uneben und abweichend von der Spindelform ist (Fig. 38).

15.15. Im Gegensatz zum oberen Teil der Zelle nimmt das Atraktosom im unteren fast die ganze Breite der Zelle ein, wo die Chromosomen den Raum anfüllen

und einige von ihnen dort nebeneinander parallel liegen (Fig. 39). Danach schreitet die Chromonematisierung der Chromosomen schnell fort.

15.45. Die Chromonematisierung einzelner Chromosomen geht hier nicht synchronistisch vor sich; sie setzt von der Peripherie der Chromosomenmasse zum Innern ein (Fig. 40).

16.12. Der untere Teil der Zelle, der der Fig. 39 entspricht, ist von erweiterten Ruhekern angefüllt (Fig. 41).

16.18. Der mittlere Teil der Zelle. Drei Vakuolen, die einige Granulen in sich enthalten, treten im Innern des Ruhekernes auf, und diese Granulen üben die BROWNsche Bewegung aus (Fig. 42).

22. Juli. Im Laufe einer Nacht werden die Vakuolen im Kern ausgeschieden. Die Ruhekernmasse gestaltet sich jetzt kugelförmig. Sowohl die Kernstruktur als auch der Zustand des Zytoplasmas sind normal und lebhafte Zytoplasmaströmungen gehen in der Zelle vor sich (Fig. 43).

29. Juli. Infolge Altersschwäche stirbt die Zelle ab.

7. Kulturversuche der Zellen in der Colchicinlösung: In Colchicinlösungen, deren Konzentrationen, geringer als bei einer 0,1-proz. Lösung sind und auf das Atraktoplasma einwirken, können die Zellen ohne Schaden zu nehmen lange leben, wie sie auch in einer 2-proz. Rohrzuckerlösung lebensfähig sind. Die Zellen mit verdoppelten Kerne, die in der Colchicinlösung kräftiges Wachstum aufzuweisen pflegen, leben oft einige Wochen lang und setzen Protoplasmaströmungen fort.

Bei den Kulturversuchen in einer Colchicinlösung stellte WALKER (1938) fest, daß das Wachstum der Zelle der Zunahme der Chromosomenzahl entspricht, jedoch stehen die beiden nicht in Proportion. Bei der Kultur der Keimwurzel verschiedener Pflanzen in der Colchicinlösung wachsen die Zellen in der Querrichtung kräftig, aber in der Längsrichtung kaum. In isolierten Staubfadenhaaren, zeigen die Zellen keine bestimmte Wachstumsrichtung. Die Zellen mit einer verdoppelten Chromosomenzahl wachsen allseitig und sehen kugelfömig aus.

Ein Ruhekern, welcher durch Colchicin-Behandlung die Chromosomenverdoppelung oder die Teilungsumkehr von einem früheren Prophasekern zum Ruhezustand durchmacht, weist bei weiterer Kultur Einbuchtungen an seiner Oberfläche auf, aber er entwickelt sich nicht zur amitotischen Teilung. Dieser Kern wechselt seine äußere Gestalt oft; sie zeigt sich in einer mehrtägigen Kultur zeitweise uneben, dann wieder glatt oder mit tiefen oder leichten Einbuchtungen. Was die Gestaltsänderung der kultivierten Kerne anbetrifft, so überzeugte ich mich davon, daß sie hauptsächlich auf den Veränderungen der Viskosität und der Oberflächenspannung bei den Kernsubstanzen beruht. Es ergibt sich auch, daß die Kolloidzustände der Kernsubstanzen den inneren und den äußeren Bedingungen für die Zelltätigkeit entsprechend, fortfahren sich zu verändern (Fig. 44–47).

Obwohl die Zellen isolierter *Tradescantia*-Staubfadenhaare in einer Colchicinlösung lange leben können, setzen die Ruhekerne ihre Kernteilung nicht von Neuem ein. Daher bin ich leider noch nicht imstande, die Verdoppelung der Chromosomenzahl bei ein und derselben Zelle wiederholt hervorzurufen und die Mitosenvorgänge bei den hohen Polyploidzellen durch Lebendbeobachtung zu beschreiben.

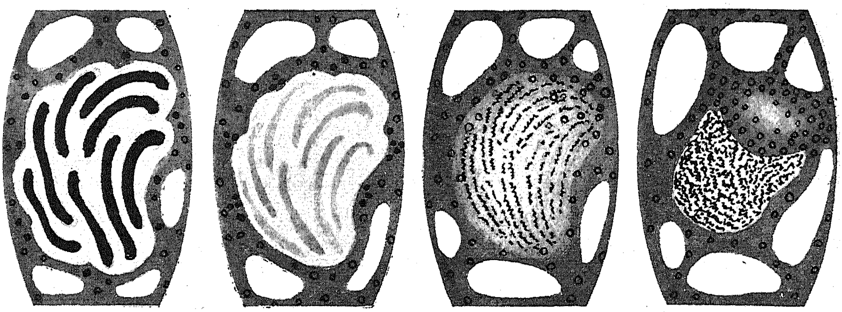
Schluß

Bei der Colchicin-Behandlung haben NEBEL und RUTTLE die Hemmung der Spindelbildung, LEVAN, SATÔ und SHIMAMURA u.a. die Untätigkeit der Spindelapparate und WALKER sowohl die Veränderung normaler Zytoplasmaorganisation als auch die Zurückhaltung der Spindelfaserbildung für Ursachen der Chromosomenverdoppelung gehalten. Ihre Erklärungen scheinen mir als morphologisch zustandegekommene Ergebnisse bei fixierten Präparaten zu gelten, aber es wird nicht genau untersucht, in welcher Weise das Colchicin die Hemmung der Spindelbildung, die Untätigkeit der Spindelapparate und die Zurückhaltung der Spindelfaserbildung verursacht.

Durch Lebendbeobachtung der C-Mitose bei *Tradescantia*-Haarzellen ist es mir jetzt gelungen, die Veränderungen der Teilungsfigur nicht nur an ihrer Morphologie sondern auch an der Veränderung ihrer Kolloidzustände kontinuierlich zu verfolgen. Durch die Ergebnisse dieser Versuche bin ich zu dem Schluß gekommen, daß die Colchicinlösung, die weniger als eine bestimmte Konzentration beträgt, unter den verschiedenen Teilungselementen auf das Atraktoplasma allein eigenartig einwirkt, aber auf andere und weiter auf die Lebensfähigkeit der Zelle kaum einen Einfluß ausübt. In dieser Eigentümlichkeit seiner Wirkung auf die Mitose unterscheidet sich das Colchicin als Auslösungsmittel polyploider Zellen von anderen Chemikalien. Das Chloralhydrat, der Essigsäuredampf, das Dampfgemisch Ammonia-Chloroform und auch das Acenaphthen können die Polyploidkerne im lebenden Zustand der Zelle hervorrufen, aber sie sind kaum in der Lage, Polyploidpflanzen zu bilden, da sie bei der Bildung polyploider Zellen nicht nur auf die Spindeltätigkeit sondern auch auf die Lebenstätigkeit der Zelle schädlich einwirken. Die Zellen können daher im Medium dieser Chemikalien nicht lange leben, solange die Chemikalien auf die Mitose wirksam sind. Dagegen fällt es auf, daß der meristematische Zustand der Zellen und der Gewebe in der Colchicinlösung lange beibehalten und die Mitosen daher wiederholt durchgeführt werden.

Was die Colchicin-Wirkung auf die Mitose anbetrifft, werden seine Wirkungen weder als Quellung des Atraktoplasmas noch als

Gelifikation desselben angenommen. Das Verhalten des Atraktoplasmas in der Colchicinlösung läßt mich vermuten, daß das Colchicin in der sich teilenden Zelle als oberflächenaktive Substanz wirkt und die Oberflächenspannung des Atraktoplasmas herabsetzt. Da die Oberfläche des Atraktoplasmas die Grenze zwischen dem Atraktosom und dem Zytoplasma bildet, gestaltet sich die Spannkraft an der Oberfläche des Atraktoplasmas als eine Grenzflächenspannung des Atraktosoms.



a b c d

Fig. 48. Schematische Darstellung der Colchicin-Wirkung auf das Atraktoplasma. **a.** Infolge der Herabsetzung der Grenzflächenspannung des Atraktoplasmas erweitert sich die Spindelfigur ungleichmäßig, aber das Atraktoplasma unterscheidet sich mit einer Grenzfläche vom Zytoplasma. **b.** Infolge der Veränderung der Chromosomenmatrix sehen die Chromosomen unklar aus. **c.** Die Chromonematisierung einzelner Chromosomen und die Zytoplasmatisierung des Atraktoplasmas treten auffallend auf. Nunmehr verflüssigt sich das Atraktoplasma, fließt zwischen den Chromosomen und häuft sich außerhalb des Restitutionskernes an. **d.** Das abgebaute Atraktoplasma bildet eine granulenreiche flüssige Plasmamasse, wo die Granulen lebhafte BROWNsche Bewegung ausüben (oben rechts am Riesenkern in der Fig.); aber diese Plasmamasse läßt sich allmählich vom Zytoplasma nicht mehr unterscheiden. Zytoplasma, zytoplasmatisierendes Atraktoplasma, Atraktoplasma, ○ sich lebhaft bewegendes Granulum, ● fast stillstehendes Granulum.

In Bezug auf die Herabsetzung der Grenzflächenspannung des Atraktoplasmas kommen wesentlich sowohl die qualitative Veränderung als auch die Viskositätsänderung des Atraktoplasmas in Frage. Diese Veränderungen werden allerdings später beim Abbau des Atraktoplasmas anschaulich, wobei sich das Atraktoplasma verflüssigt und zytoplasmatisiert. Verschiedene morphologische Veränderungen bei der C-Mitose können in der Tat als Resultat der Herabsetzung der Grenzflächenspannung des Atraktoplasmas und als deren Begleiterscheinungen erklärt werden.

Erstens: Das Erweitern des Atraktosoms und seine unebene Kontur ergeben sich aus der Herabsetzung der Grenzflächenspannung des Atraktoplasmas; in fixierten Präparaten stellt man solche

Zustände als Verschwinden der Spindelfigur oder als das der Spindelfasern fest.

Zweitens: Das Zerstreuen der Chromosomen im erweiterten Atraktosom, die Streckung der Chromosomen, die Verminderung der Chiasmata, die Erleichterung der Chromosomenspaltung und die C-Paarung der Halbchromosomen—alle diese Erscheinungen werden als Folge der Herabsetzung der Grenzflächenspannung inner- und außerhalb des Atraktoplasmas angenommen, da die Grenzflächenspannung im Innern des Atraktosoms auch auf die Grenzfläche einzelner Chromosomen einen Druck ausübt. Durch Verminderung dieses Druckes werden daher einzelne Chromosomen in einen neuen Gleichgewichtszustand gebracht und bleiben nunmehr außerhalb der Regulierung der normalen Spindeltätigkeit. Der neue Gleichgewichtszustand dauert bis zum Einsetzen der Chromonematisierung einzelner Chromosomen, aber die Teilung der Centromeren allein wird durch die Veränderung der Außenkraft kaum beeinflußt, daher geht ihre Trennung unabhängig von anderen Teilungselementen vor sich.

Drittens: Bei einem Vergleich der Abnahme der Grenzflächenspannung des Atraktoplasmas bei der Colchicin-Behandlung mit der Zunahme derselben bei der Behandlung mit dem Dampfgemisch Ammonia-Chloroform, fällt es auf, daß das Atraktoplasma im letzteren Fall anquillt und seine Grenzfläche sich allseitig glatt ausprägt (siehe WADA 1939b, S. 164 Textfig. 9). Die Spannkraft, durch welche der angequollene Zustand des Atraktosoms beibehalten wird, übt auch im Innern des Atraktoplasmas auf die einzelnen Chromosomen einen Druck aus. Daher können die Chromosomen im angequollenen Atraktoplasma nicht mehr voneinander gelöst bleiben; sie treffen miteinander zusammen und bilden einen Chromosomenklumpen (WADA 1939b). Dieses Verhalten der Chromosomen dient als ein entgegengesetztes Beispiel für das Verhalten der Chromosomen bei der Colchicin-Behandlung, wobei alle Chromosomen infolge der Abnahme der von außen wirkenden Kraft voneinander gelöst bleiben.

Im Gegensatz zu den morphologischen Veränderungen des Atraktoplasmas bei der Colchicin-Behandlung, welche als Folge der Herabsetzung der Grenzflächenspannung des Atraktoplasmas angenommen werden, bin ich gegenwärtig noch nicht imstande, diese Veränderungen physikochemisch experimentell nachzuweisen. Hinsichtlich der physikochemischen Untersuchungen über das Atraktoplasma bleiben noch viele Probleme zukünftigen Experimenten vorbehalten.

Die Wirkung des Colchicins auf die Spindelfigur ist schon von allen Forschern anerkannt, aber seine Wirkung auf die Prophase-

kerne ist noch nicht restlos geklärt. Aus den Ergebnissen oben erwähnter Lebendbeobachtungen bei den *Tradescantia*-Haarzellen ergibt sich, daß die Chromosomenverdoppelung auch am Ende der Prophase einsetzt und ohne Eintritt in die Metaphase vollendet wird. Die Chromosomenverdoppelung bei solchen Teilungsstadien bezieht sich hauptsächlich auf die Veränderung des Atraktoplasmas selbst, und geht im Innern des Prophasekernraumes vor sich.

Die Polyploidisierung ohne Metaphasespindel ist dem Verhalten der mitotischen Kerne bei der, von GEITLER (1939) bei Wanzen untersuchten Endomitose äußerst ähnlich. Bei den in der Colchicinlösung lang behandelten meristematischen Geweben muß die Polyploidisierung der Zellkerne vermutlich auch endomitotisch wiederholt durchgeführt werden. Weiter vermute ich, daß die Endomitose irgend eines Wirkstoffes, der wie das Colchicin unter verschiedenen Teilungselementen allein auf das Atraktoplasma eigenartig einwirkt, bedarf, daß ein solcher Wirkstoff in bestimmten ontogenischen Stadien in bestimmten Geweben physiologisch oder durch gesteigerte trophische Funktion sekletiert wird, und daß die Polyploidisierung, solange der Wirkstoff im Gewebe vorhanden ist, in dem Prophasekern durch die Entwicklung der Schwesterchromatiden zu einzelnen Chromosomen wiederholt vor sich geht.

Wie wiederholt erwähnt, konstatierte ich unter der Colchicin-Wirkung die kontinuierliche Gestaltsänderung der Spindelfigur und das Vorhandensein des Atraktoplasmas in der späten Prophase, Metaphase, Anaphase und auch in der Telophase als Phragmoplast-Substanz, wobei sich das Atraktoplasma und auch die Phragmoplast-Substanz, ungeachtet der Herabsetzung ihrer Grenzflächenspannung, durch eine Grenzfläche vom Zytoplasma unterscheiden und das erstere die Chromosomen umgibt. Der Abbau des Atraktoplasmas und der Phragmoplast-Substanz, infolgedessen ihre Vermischung mit dem Zytoplasma, setzen parallel mit der Chromonematisierung der Chromosomen ein und diese zytoplasmatisieren sich.

Bei der Zytoplasmatisierung sieht die Grenze des Atraktoplasmas mit den Chromosomen und mit dem Zytoplasma zuerst unklar aus, dann verflüssigt sich das Atraktoplasma und Fällungen treten oft in ihm als winzige Granulen auf; es entwickelt sich schließlich zur granulenreichen flüssigen Plasmamasse und fließt mit dem Zytoplasma der Mutterzelle zusammen. Die Zytoplasmatisierung des Atraktoplasmas in der späten Prophase oder in der Metaphase und Anaphase, und weiter diejenige der Phragmoplast-Substanz in der Telophase gehen wesentlich in derselbsen Weise vor sich.

Zum Schluß bin ich imstande zu behaupten, daß das Verhalten des Atraktoplasmas unter der Colchicin-Wirkung, hinsichtlich der Atraktoplasma-Theorie, den Ergebnissen anderer Versuchen nicht widerspricht, die durch Anstichversuche oder durch Versuche mit dem Dampfgemisch Ammonia-Chloroform oder mit anderen Substanzen festgestellt worden sind.

Zusammenfassung

Durch Lebendbeobachtungen wurde die Einwirkung des Colchicins auf die Mitose der Staubfadenhaarzellen von *Tradescantia* untersucht, wobei das Verhalten der Spindelfigur nicht nur an ihrer Morphologie, sondern auch an der Veränderung ihrer Kolloidzustände kontinuierlich verfolgt wird.

Das Colchicin wirkt unter verschiedenen Teilungselementen allein auf das Atraktoplasma eigenartig, aber auf andere und weiter auf die Lebensfähigkeit der Zelle kaum ein.

Bei dem Verhalten des Atraktoplasmas in der Colchicinlösung ist angenommen, daß das Colchicin in der sich teilenden Zelle als oberflächenaktive Substanz wirkt und die Grenzflächenspannung des Atraktoplasmas herabsetzt. Verschiedene Veränderungen bei der C-Mitose können als Resultat der Herabsetzung der Grenzflächenspannung des Atraktoplasmas und als ihre Begleiterscheinungen erklärt werden.

Unter der Colchicinlösung setzt bei dem sich teilenden Kerne schon in der späten Prophase die Chromosomenverdoppelung ein, welche ohne Eintritt in die Metaphase vor sich geht.

Ungeachtet der Veränderung des Atraktosoms bei der C-Mitose unterscheiden sich das Atraktoplasma und auch die Phragmoplast-Substanz durch eine Grenzfläche vom Zytoplasma und das erstere umgibt die zerstreuten Chromosomen. Der Abbau des Atraktoplasmas setzt parallel mit der Chromonematisierung der Chromosomen ein und das Atraktoplasma zytoplasmatisiert sich.

Bei der Zytoplasmatisierung verflüssigt sich das Atraktoplasma, entwickelt sich schließlich zur granulenreichen, flüssigen Plasmamasse und fließt mit dem Zytoplasma der Mutterzelle zusammen.

Zum Schluß spreche ich der Japanischen Gesellschaft zur Förderung der Zytologie für die finanzielle Unterstützung dieser Arbeit meinen besten Dank aus.

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Fakultät der Kaiserl. Universität Tokyo

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Nachschrift: Nach Abschluß des Manuskriptes dieser Mitteilung ist eine Abhandlung von GEITLER erschienen (Die Polyploidie der Dauergewebe höherer Pflanzen. Ber. d. Deut. Bot. Gesell. 58 : 131-142. 1940). In dieser Abhandlung wird festgestellt, daß die Polyploidisierung bei einigen Pflanzen durch Vervielfachung der Chromosomen im Ruhekern (innere Teilung) erfolgt. Nunmehr handelt es sich bei der inneren Teilung um die Chromosomen im Ruhekern. Der Kern, in welchem sich der Kerninhalt zu Chromatinfäden oder weiter zu Chromosomen entwickelt hat, wird nicht mehr als ein Ruhekern angesehen, sondern er befindet sich bereits in einem mitotischen Vorgang. Die innere Teilung scheint mir daher auch eine stark ausgelassene abnorme Mitose zu sein, in welcher die Polyploidisierung, wie die durch die Colchicin-Wirkung im Prophasekern induzierten Chromosomenverdoppelungen, vermutlich auch in der späten Prophase infolge einer Sekretion irgend eines Wirkstoffes durch Veränderungen des Atraktoplasmas erfolgt.

Contributions to the Cytology of Genus *Saccharum*

I. Observations on the F_1 progeny of sugar cane-sorghum hybrids

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Following the success of attempts in India to obtain early-maturing sugar cane varieties by crossing sugar cane with sorghum (Thomas and Venkatraman 1930, Venkatraman and Thomas 1932), similar attempts at crossing have been made with success in the United States (Bourne 1935), in India (Singh 1934 a, b, Janaki Ammal and Singh 1936), and at our Government Sugar Experiment Station, Tainan, Taiwan (Formosa). From such matings, certain desirable early lines of sugar cane have been already obtained in India (Venkatraman and Thomas 1932, Viswa Nath, Ramasubrahmarrya Ayyar and Varahala 1934). Although at our Station, no economical varieties have yet been produced, owing to the short period since this work was begun, the outlook for the future, however, is promising.

Some of the results of our breeding experiments have already been published by Yamasaki and Nakamura (1935 a, b), and Nakamura (1937), and the writer's cytological studies were reported as a preliminary note (1938).

In the F_1 generation of sugar cane-sorghum hybrids, we obtained three types, the first greatly resembling sugar cane in its external characters, which we shall call the normal, or sugar cane type, while the second type is very short in height compared with the common sugar canes, most of them presenting a bushy appearance. This will be called the dwarf, or terato-type (Fig. 1). The most notable fact with our F_1 generation is the high proportion (60–70%) in which these dwarf plants occur. Some of them were dwarfed to an extraordinary degree, with very short stems that in some cases could scarecely be noticed as stems. In these plants, only the leaves could be seen. They all died before November of the first year of experiments, preceding maturation (cf. Yamasaki and Nakamura 1935 a, b, Nakamura 1937). The third, or last, type of the F_1 was intermediate between the above mentioned two types, some individuals being sprout tillers, as in sugar cane, although their appearance was still abnormal. This will be called the intermediate

type. In each of the dwarf and intermediate types, certain differences could be seen in their external characters among different individuals.

In 1934, the proportion of these three types was 13.5%, 62.9%, and 23.6% respectively (Yamasaki and Nakamura 1935 a). But the proportion of the dwarf type varied with the variety of sorghums used as the male parent, and also with the particular year of experiment (Nakamura 1937).


Fig. 1. F_1 of dwarf type. 2725 POJ×*Sorghum vulgare*. a, 2725 POJ×Hei ko shuang hsin pan (a Kaoliang from Manchoukuo). In the background, an F_1 plant of normal type is seen. b, 2725 POJ×Karum Cholam. This plant died before the middle of November 1938. Scale in centimeter. Photo. Sept. 3, 1938.

This paper is concerned with the cytological observations made on the above mentioned three types of hybrids, with special reference to their chromosome constitution as well as to the meiotic behaviour of the normal and the intermediate types.

Material and method

The crossings were made at our Cane Breeding Division, and the F_1 plants raised from the resultant seeds, or the plants that were vegetatively propagated from them were used as material. The combinations of parents in the crosses are shown in Table 1. Besides these, a fixed material of an F_1 plant, belonging to our intermediate type, which was sent to us from the Saipan Branch of the Tropical Industry Institute, South Seas Bureau, Saipan, Micronesia, was studied for comparison.[1] The last named plant is reported to be ever flowering through the year.

1) I wish to express here my hearty thanks to Mr. I. YAMANAKA, Chief of the Branch, who kindly supplied me with this material.

Table 1. Materials for F_1

Types of F_1	Lines	Parentage ♀	Parentage ♂	Fixing date of PMC
Normal type (sugar cane type)	3/9C	2725 POJ ×	Akamoti (a sorghum variety from Dept. of Agr., Gov. Res. Inst. Formosa, Japan)	
	19/9C	2725 POJ ×	Sweet sorghum (from Tokusima Prefecture)	Dec. 1938
Intermediate type	10/11C	2725 POJ ×	Sweet sorghum (from Tokusima Prefecture)	Dec. 1937
	13/11C	2725 POJ ×	A variety of sorghum (from Kagawa Prefecture)	Nov. 1937
	15/11C	,, ×	,,	,,
	16/11C	,, ×	,,	March 1937
	73/11C	2725 POJ ×	Hung ku chin sui huang (a Kaoliang from Manchoukuo)	Nov. 1937
	Ever flow. F_1 (Saipan)	2725 POJ ×	A variety of Sweet sorghum	Dec. 1938
Dwarf type (terato-type)	13C-A	2725 POJ ×	Hei ko shuang hsin pan (a Kaoliang from Manchoukuo)	
	13C-B	2725 POJ ×	A variety of Broom corn	
	13C-C	,, ×	,,	
	13C-D	2725 POJ ×	Huan kan kaoliang (a Kaoliang from Manchoukuo)	
	13C-E	2725 POJ ×	Karum Cholam (a variety of sorghum from India)	
	13C-F	2725 POJ ×	Sweet sorghum (from Tokusima Prefecture)	
	13C-G	2725 POJ ×	Hung ku chin sui huang (a Kaoliang from Manchoukuo)	

In the case of lines marked 9C, the crosses were effected in the winter of 1933, and those marked 11C and 13C were obtained from crosses made in the winter of 1935 and 1937 respectively.
All plants of dwarf type died before flowering.

In addition to the foregoing, the roots tips and the inflorescences of a sugar cane variety 2725 POJ that was used as the female parent, were fixed in the autumn of 1935. The chromosome numbers of the grain sorghum varieties that were used as male parents were examined in meiosis.

The root tips were fixed in Flemming's weak and strong solutions, Flemming's solution of Bonn, Allen's Bouin, Benda's solution, and Navashin's fluid. The fixatives corresponding to the various figures are: for Fig. 2, Flemming's weak solution; for Figs. 8, 9, 19, 20, 22, and 24, Flemming's solution of Bonn; for Fig. 21, Allen's Bouin; for Fig. 23, Benda's solution; for Fig. 25, Navashin's fluid; and for Figs. 26–28, Flemming's strong solution. The fixed materials were imbedded in paraffin, cut 15 μ thick, and stained with Heidenhain's iron-alum haematoxylin. So far as these materials are concerned, Flemming's solution of Bonn gave the best results, but it is necessary, although at the same time it is difficult, to find the most

suitable fixative for the sugar cane. Of the varieties or lines that were fixed at various times with various fixatives, the results diverged very widely.

Observations of reduction division were made exclusively in PMC, fixed in Carnoy's fixative. The spikelets of 2725 POJ were imbedded in paraffin, cut and stained the same as the root tips. Fixed spikelets of sorghums and F_1 were observed, after being preserved in 70% alcohol, in Belling's iron-acetocarmine. This showed good figures.

Figures were drawn on a drawing table with the aid of an Abbe's camera, using Zeiss ocular K 15 × and objective × 120. The original magnification was 3150 times, which for most figures was reduced in representation to the respective magnifications shown with each figure.

Observations

I. Female parent, sugar cane variety 2725 POJ

A) *Somatic chromosomes.* The number was proved to be $2n = 107$ (Fig. 2), which agrees with those found by Bremer (1928) and Yamashita (1937). Many long chromosomes, constricted at the median or submedian positions, were observed. Chromosomes that are constricted at two or three points were also observed. Although the differences in the lengths of the chromosomes were considerable, classification of them all according to their morphological characters is hardly feasible. In plants that are largely of a hybrid nature, like the sugar cane, it is very necessary to know the number of somatic chromosomes in the root tip cells before attempting a study of their meiosis.

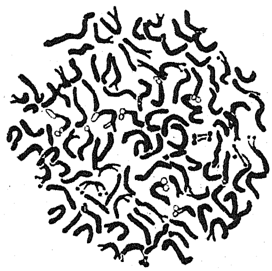
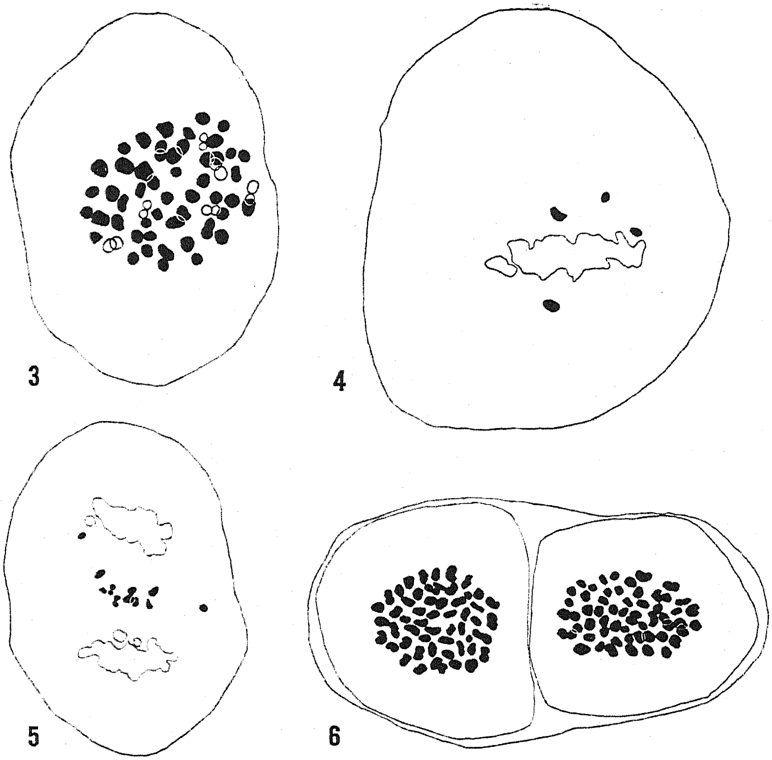


Fig. 2. Somatic chromosomes of 2725 POJ. $2n = 107$. ×3150.

B) *Meiosis.* In the polar view of the heterotypic metaphase in PMC, 52–69 chromosome elements were counted, including uni-, bi- and, occasionally, multivalent chromosomes (Fig. 3). At diakinesis, 50 chromosomes were observed which may be owing to the multiple conjugation of chromosomes. At the metaphase, some of the univalents must be the separated halves of bivalents, while some are so from the beginning. It is difficult to identify by mere size the univalents of the latter category among the bivalents. Such univalents, however, may be clearly recognized in the side view of

the metaphase (Fig. 4). At the anaphase of heterotypic division, about 10 univalents were observed lagging behind, which passed to both poles after splitting lengthwise, or without doing so (Fig. 5). Bremer (1928) observed, at the anaphase of the heterotypic division in PMC of this variety, 106 or 107 chromosomes, including a few lagging univalents. He also counted 57 chromosomes at the heterotypic metaphase, although he has given no description of the homotypic division.



Figs. 3–6. Meiosis of 2725 POJ. 3, polar view of heterotypic metaphase. The two chromosomes composing a pair and shown in white are the halves of a bivalent; 56 chromosomes. 4, side view of heterotypic metaphase, univalents are observable. 5, side view of heterotypic anaphase, some univalents are split, while others are not. 6, polar view of homotypic metaphase. In the right nuclear plate, ca. 58, and in the left, ca. 54 chromosomes are counted. ×2500.

In my material, 49–58 chromosomes were counted in the metaphasic polar view of the homotypic division (Fig. 6). This variation in the chromosome number may be owing to the behaviour of the univalents during heterotypic division. In the tetrad stage, most PMC were found to have formed triads, true tetrads occurring but rarely. The pollen grains were completely sterile.

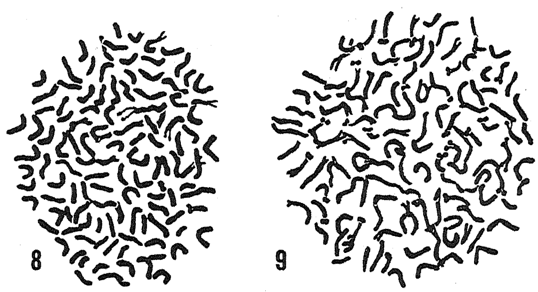
II. Male parents, sorghum varieties

In all the male parents that were used in the present study, the number of chromosomes was n =10, and neither polyvalent chromosomes nor meiotic irregularities were detected, which agrees with my previous results (1936), so that only one figure of the chromosomes of Sweet sorghum (From Tokusima Prefecture) is shown here (Fig. 7).

Fig. 7. Haploid chromosome set from Sweet sorghum (from Tokusima Prefecture). ×1600.

III. F_1 of normal type

A) *Somatic chromosomes.* The 2 F_1 plants of lines 3/9C and 19/9C showed 2n =118 (Figs. 8 and 9). Notwithstanding that the chromosomes of sugar cane and sorghums are so small and slender that accurate counting is sometimes very difficult, in the best plates as many as 118 chromosomes were clearly counted. In the root tip cells of F_1 plants, parental chromosomes could scarcely be distinguished morphologically from one another.

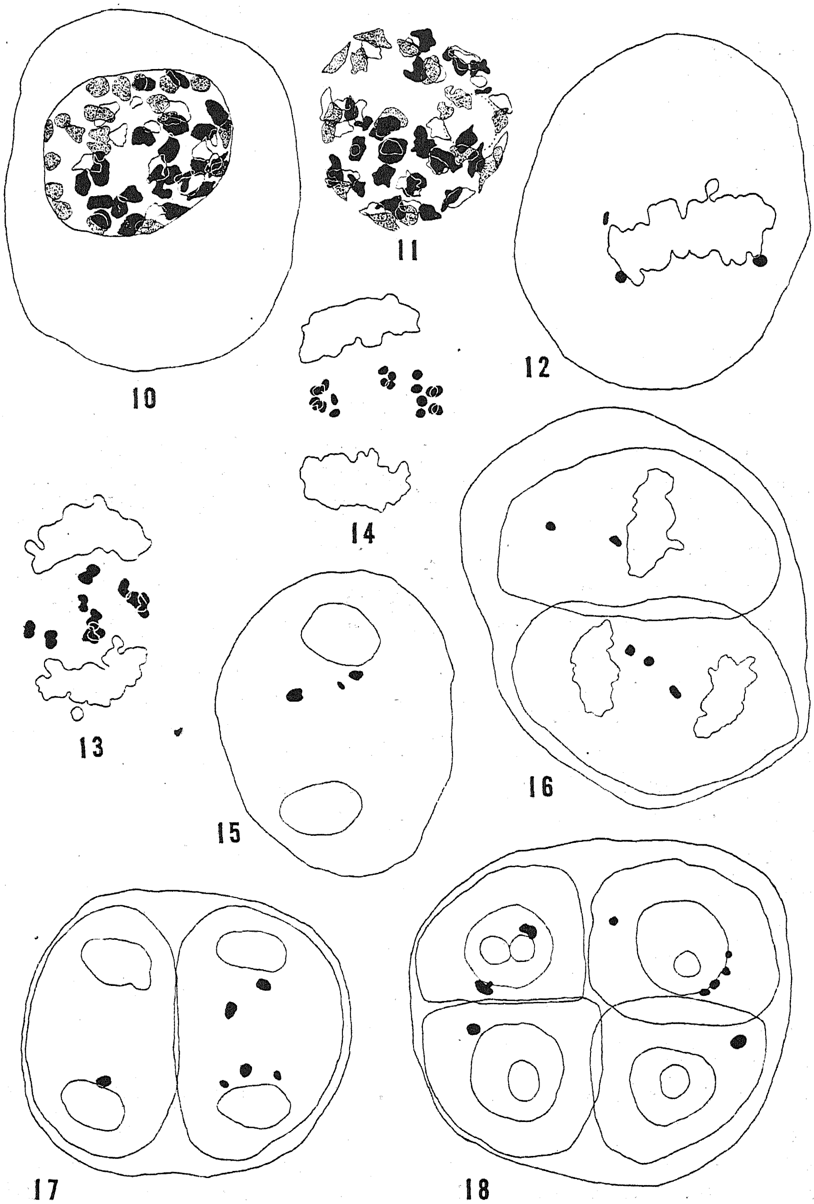


Figs. 8, 9. Somatic chromosomes of 2 F_1 plants of normal type. 2n = 118. 8, 3/9C. 9, 19/9C. ×3150.

These two lines may have, in their somatic cells, double sets of maternal chromosomes, as in Bremer's F_1 *Saccharum officinarum* (n = 40) × *Sacc. spontaneum* (n = 56) (1923, 1928), Dutt and Rao's F_1 Vellai (*Saccharum officinarum*, n = 40) × *Sacc. spontaneum* (from Coimbatore, n = 32) (1933), and Singh's F_1 2725 POJ (2n = 106–107) × *Sorghum Durra* (n = 10) (1934 b).

B) *Meiosis.* Observations were made only in an individual of 19/9C. In 6 PMC, in diakinesis, about 52–62 chromosome elements were counted (Fig. 10). In one PMC, 59 chromosomes, exactly half the somatic number of this line, were found in diakinesis (Fig. 11), but not all of them were bivalents, the smaller ones being recognized as univalents. Thus, some of the large chromosome elements may probably be tri- or polyvalents. The existence of univalents was also ascertained without doubt in the side view of the metaphase (Fig. 12). In the heterotypic anaphase and early telophase, univalents were always found lagging, the number of which,

in 15 PMC, was 6–12. Of these numbers, 10, and nearly 10, were most frequently (in 10 PMC) observed (Figs. 13 and 14). In the PMC, in which numbers other than 10 were counted, the arrange-



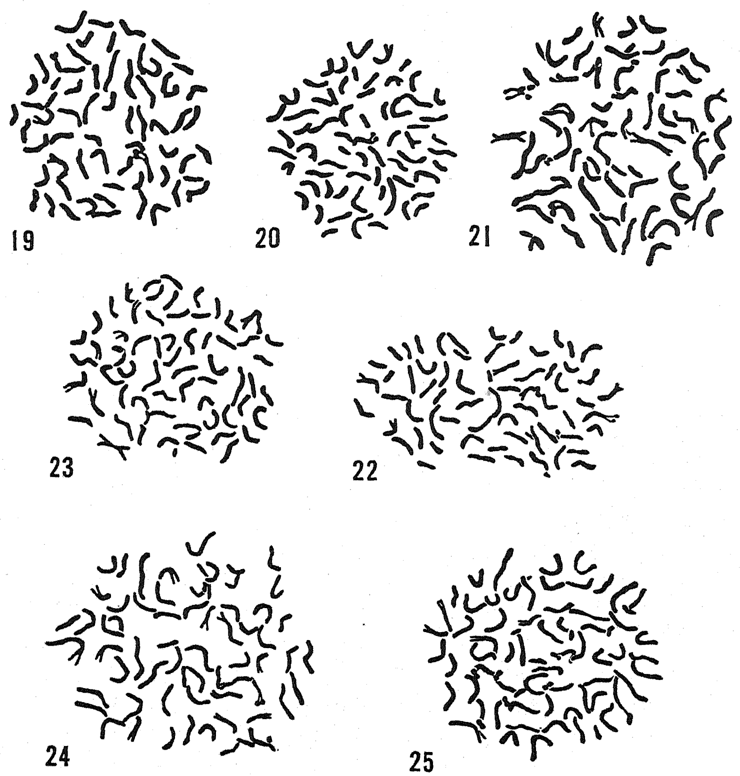
Figs. 10–18. Meiosis of 19/9C, normal type of F_1. **10**, diakinesis, ca. 53 chromosomes. **11**, ditto, 59 chromosomes. **12**, heterotypic metaphase. **13, 14**, heterotypic anaphase showing 10 univalents. **15**, heterotypic telophase. **16**, homotypic metaphase and anaphase. **17**, homotypic telophase. **18**, pollen tetrad. ×1500.

ment of chromosomes was unfavourable for the counting. So that *the number of univalents in this plant may be recognized as 10.* The laggards left behind were usually observed at the telophase (Fig. 15).

In homotypic metaphase or anaphase, chromosomes that were located apart from the equatorial plate or were lagging behind were found, as in heterotypic division (Fig. 16). Fig. 17 shows the telophase of homotypic division. Although the pollen tetrad was usually formed, extra nuclei were very frequently found (Fig. 18). The pollen grains were completely sterile.

IV. F_1 of dwarf type

In 7 individuals, each belonging to different lines, the chromosome numbers were found to be exactly $2n = 64$ (Figs. 19–25). The number $2n = 65$ was sometimes observed in the same preparations,



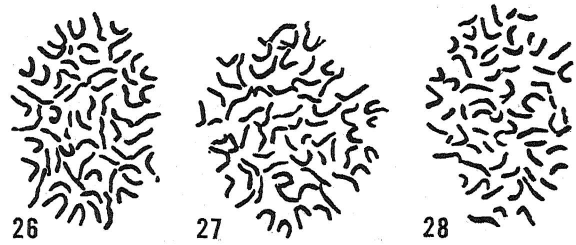
Figs. 19–25. Somatic chromosomes of 7 F_1 plants of dwarf type. $2n = 64$. **19**, 13 C–A. **20**, 13 C–B. **21**, 13 C–C. **22**, 13 C–D. **23**, 13 C–E. **24**, 13 C–F. **25**, 13 C–G. ×3150.

probably on account of the fragmentation. As in F_1 of normal type, identification of parental chromosomes was scarcely possible. In

these lines, all the individuals died before inflorescence could be obtained for studying meiosis.

V. F_1 of intermediate type

A) *Somatic chromosomes.* In 3 lines, namely, 13/11C, 15/11C, and 73/11C, 2n = 64 was found to be the number (Figs. 26–28). In these lines, numbers that deviated from this were often observed in the same preparations, owing probably to the unfavourable arrangement of chromosomes. The number 2n = 65 may possibly be the result of fragmentation. In the finest metaphasic plates, however, I counted, in most cases, 64 chromosomes. In a plant of 16/11C also, 2n = ca. 64 was observed.



Figs. 26–28. Somatic chromosomes of 3 F_1 plants of intermediate type. 2n = 64. 26, 13/11 C. 27, 15/11 C. 28, 73/11 C. ×3150.

The four lines above described were derived from the crosses made in 1935, the morphological characters differing with different lines. A curious line is 16/11C, having very short stems reaching to a height of about a meter, soon dying off, with other new stalks sprouting from the parts underground. The stalks of this plant had hardly any bud eyes (cf. Nakamura 1937, Fig. 8). Although in March 1937, this line sent out inflorescences that were completely male sterile, no material for studying meiosis could be obtatined, and no inflorescence could be seen thence to the present.

B) *Meiosis.* The meiotic behaviour of 3 plants, belonging to each of lines 13/11C, 15/11C, and 73/11C, were about the same.

Table 2. Frequency of the number of chromosome elements in diakinesis and in the heterotypic metaphase of F_1 of intermediate type

Lines	Number of chromosome elements																			Number of PMC observed
	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	47	49	
73/11C		1		1	1	1	3	1	2	6	3	2	1		1					23
15/11C								2	3	1	1		1			1				9
13/11C								1	2	3			1	1	1					9
10/11C												1					2	1	1	5
Ever flow. F_1 (Saipan)	1				1			1		3		1								7
Total	1	1	0	1	2	1	3	5	7	13	4	4	3	1	2	1	2	1	1	53

At diakinesis or in the polar view of the heterotypic metaphase, most frequently 37 chromosome elements were counted, although a rather wide variation was observed, as shown in Table 2. While these chromosome elements were usually bivalents or univalents, sometimes they were trivalents, quadrivalents, and even polyvalents (Figs. 29 a, b, c).

The number of univalents, in many cases, exceeded 10. In Fig. 30 a large polyvalent chromosome is seen. Such large polyvalents were found thrice in the course of my observations. In line 73/11C, I found in most cases 11–16 univalent chromosomes in the side views of the heterotypic meta-, ana- and telophases, while up to 20 univalents were observed, although rarely, as shown in Table 3.

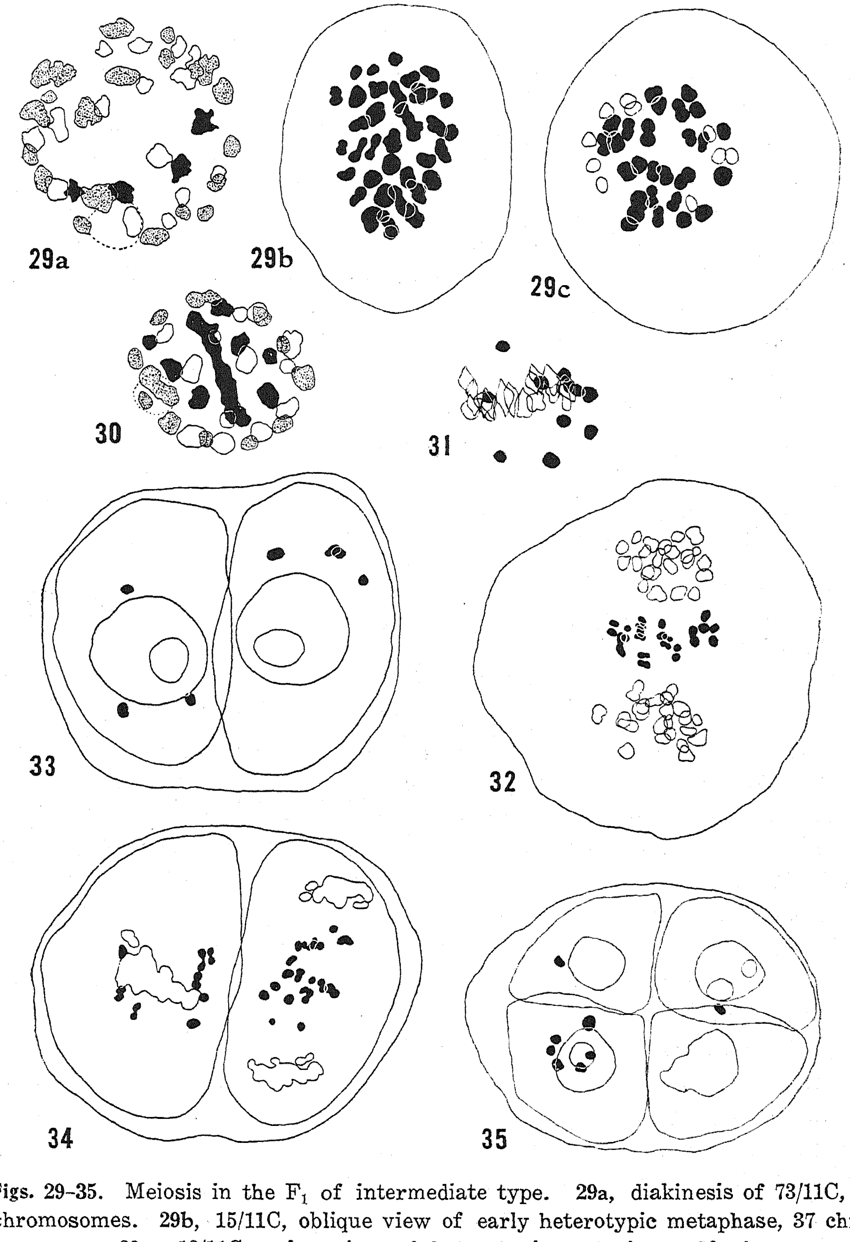
Table 3. Univalent frequency in 73/11C

Phases	Number of univalents											Number of PMC observed
	10	11	12	13	14	15	16	17	18	19	20	
Metaphase	6	9	8	9	7	4	3	1	2	2	0	51
Anaphase—early telophase	0	3	7	4	3	2	7	3	5	6	3	43
Total	6	12	15	13	10	6	10	4	7	8	3	94

In 13/11C and 15/11C, chromosome behaviour, similar to that just described, were observed. But in 10/11C, the numbers of univalents were somewhat larger, 17–21 being found in the metaphase or anaphase. In this line, the number of chromosome elements in PMC was slightly more than that in other lines, namely, 39–49 in diakinesis or in heterotypic metaphase (Table 2), which may have some bearings on the fact that its stem length exceeded that of other F_1 plants of intermediate type. Further studies are, however, necessary for elucidating this point.

These univalents were distinguished fairly clearly from bivalents, seeing that the former were either round or elliptical, and smaller, while the latter were of diamond-, dum-bell-, or short-armed cross-shapes, and larger (Fig. 31). In some cases, these univalents arranged themselves on the equatotial plane, but in others they were distributed away from this plane. During the anaphase, they were found lagging and all split (Fig. 32).

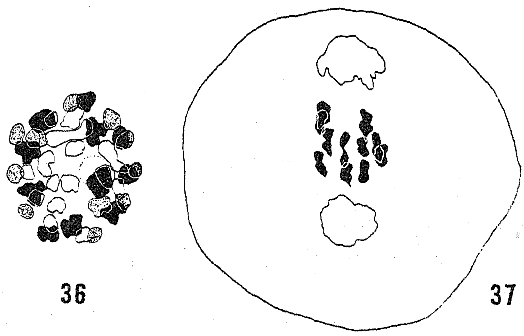
At interkinesis, extra chromatic clumps were often observed in the cytoplasm (Fig. 33). In homotypic division, the laggards were found again at anaphase (Fig. 34), where they were sometimes cut at the point of constriction, and the halves going to opposite poles. The pollen tetrads usually consisted of 4 cells, but shrinking of cells,



Figs. 29–35. Meiosis in the F_1 of intermediate type. 29a, diakinesis of 73/11C, 37 chromosomes. 29b, 15/11C, oblique view of early heterotypic metaphase, 37 chromosomes. 29c, 13/11C, polar view of heterotypic metaphase, 36 chromosomes. Those shown in white are probably univalents. 30, 73/11C, large polyvalent chromosome in diakinesis. 31, 73/11C, side view of heterotypic metaphase, 11 univalents. Bivalents are not all drawn. 32, 73/11C, heterotypic anaphase. 12 univalents are lagging and split. 33, 73/11C, interkinesis. 34, 73/11C, homotypic division. Univalents lagging at the anaphase are much more numerous than in the heterotypic division. 35, 73/11C, pollen tetrad. ×1500.

nuclei of abnormal shape, and chromosomes located outside the nuclei were observed (Fig. 35). The pollen grains were sterile in all the lines examined.

The ever flowering F_1 of intermediate type received from Saipan Island is said to attain to a height of about 60 cm. In the preparations of root tips of this line, the chromosome numbers did not differ much from 64, although the exact number could not be made out. In 7 PMC in diakinesis, 28–39 chromosomes were counted. I have met with the PMC showing 37 chromosomes three times (Table 2. Fig. 36). The numbers of univalents were 10–15 in side views of the heterotypic metaphase, anaphase, and early telophase, the number most frequently observed being 11 (Fig. 37). These numbers are about the same as in 73/11C. Thus, in this line, 2n = 64 might also be expected. Although in the pollen tetrad stage 4 cells were commonly observed, the number of chromosomes left outside the nuclei was considerable. In this material, the pollen grains were completely abortive.



36 37

Figs. 36, 37. Meiosis of an ever flowering F_1 of intermediate type from Saipan. 36, diakinesis, 37 chromosomes. 37, late anaphase of heterotypic division, 11 univalents. ×1500.

Discussion

In many cases of meiosis of the sugar cane variety 2725 POJ, we observed bivalent and univalent chromosomes, and a small number of multivalents. Huskins and Smith (1934) wrote regarding the meiosis of sorghums that "In all the diploid forms of sorghum examined, 10 bivalents are most commonly formed, but quadrivalent associations are also common and sexivalents are found occasionally". But, in the sorghum parents used by the writer in the present study, no polyvalents were formed in their PMC. The same was the experiences of the writer (1936) and Ishihara (1937) with Manchurian Kaoliangs (*Sorghum vulgare* vars.), the bivalent chromosomes being always normally formed, and neither have other authors found any polyvalent conjugation in sorghum.

A normal type of F_1 of the sugar cane-sorghum hybrid, 19/11C, showed in the meiosis of PMC about 10 lagging chromosomes. These

may have been derived mainly from the sorghum parent, judging from the results just mentioned, but from the observations of Huskins and Smith cited above, some of the univalents found in my normal type of F_1 may be regarded as having been derived from sugar cane. At the same time, the probability of allosyndesis in this F_1 may not be out of question. But it seems most reasonable to regard the somatic chromosome number 118 of this F_1 as being the result of the fusion of the haploid sorghum chromosomes and the double sets of reduced chromosomes of 2725 POJ, and the univalents found in this F_1 may have originated mainly from sorghum.

In the intermediate type of F_1, 10 lagging chromosomes may have originated from sorghum, while the other laggards may have come from sugar cane. It may therefore be said that the chromosomes derived from sugar cane in the intermediate type of F_1, formed a large number of bivalents, and these gemini which, in the side view of the heterotypic metaphase, are mostly diamond-, dum-bell-, or short-armed cross-shaped (Fig. 31), point to chiasma evidence among the reduced chromosomes of the sugar cane. In other words, *a large number of the members of the chromosomes in the somatic cells of 2725 POJ that were used as the female parent were of quadruple nature.*

This assumption of autosyndesis in sugar cane chromosomes is also supported by the history of the formation of 2725 POJ, which is shown, according to van Deventer (1927) and Bremer (1923, 1924, 1928), in Fig. 38, from which it will be seen that 40 chromosomes of Black Cheribon and also the same number of chromosomes out of 89 in 100 POJ are assumed to have doubled in the formation of Kassoer and 2364 POJ, respectively. In this connection it may be cited that, in autohexaploid *Fragaria elatior* ($n=21$), 21_{II} were always found (Kihara 1926), and that in the F_1 formed between this and a diploid species *F. nipponica* ($n=7$), 14_{II} were usually observed (Lilienfeld 1933, 1936). In this example also, the occurrence of auto- and allosyndesis in the F_1 is shown.

We have examples in the genus *Saccharum* in which bivalent chromosomes were unexpectedly formed. Bremer (1923, 1928) observed in the F_1 ($2n=136=40+40+56$) produced by *Saccharum officinarum* ($n=40$) $\times$ *Sacc. spontaneum* ($n=56$), mostly bivalent chromosomes; which conjugation he explained by the allosyndesis of 56 chromosomes of each parent and the autosyndesis of 24 chromosomes in *officinarum*. Dutt and Rao (1933) counted 56_{II} at the heterotypic metaphase of PMC of the variety Co. 205, which is an F_1 progeny formed by crossing Vellai ($n=40$,

a variety of *Saccharum officinarum*) with *Sacc. spontaneum* from Coimbatore ($n = 32$). This line is said to have double sets of maternal chromosomes $\left(n = 56 = \frac{40+40+32}{2}\right)$, so that it might be thought that the 80 chromosomes from the female parent formed 40_{II}, and that autosyndesis occurred in 32 paternal chromosomes, although allosyndesis might not be entirely excluded. Meiotic division was reported to have proceeded almost normally.

Singh (1934 a) found 34 chromosome elements, including univalents and bivalents, at the heterotypic metaphase of PMC of the hybrid Vellai $\times$ *Saccharum Narenga* ($n = 15$). It might be supposed that the chromosome configuration observed by him was $1_{IV} + 18_{II} + 15_{I}$, which was derived from $20_{II} + 15_{I}$. According to Bremer (1925), in *Saccharum Narenga,* 15_{II} always appeared at diakinesis, while at the stages of pollen tetrad and pollen formation, no irregularities were observed. From comparative studies of the size of the nuclei in meiosis, he concluded that this species is not a genuine *Saccharum,* whence the probability of allosyndesis in Singh's F_1 may be small, although not entirely excluded.

Singh (1934 a) observed 39_{II} at the heterotypic metaphase of PMC in the hybrid Saretha ($2n = 92$, belonging to *Saccharum barberi* Jeswiet) $\times$ *Sacc. spontaneum* from Coimbatore ($n = 32$). Regarding the chromosome conjugation of this hybrid, no explanation was given. In the F_1 hybrid of 2725 POJ ($2n =$ ca. 106) crossed with *Sorghum Durra* ($n = 10$), owing to the doubling of the maternal chromosomes, he also observed 58 chromosomes that were believed to be bivalents. In the example last cited, however, it may not be proper to suppose that all the 58 chromosomes were bivalents, seeing that in my normal type of F_1, a certain number of univalents were usually observed, although in one case half the somatic number was exactly counted, and that the meiosis in this hybrid was reported to be very irregular as a rule.

Ljungdahl (1924) was the first to find true auto- and allosyndesis. According to her, F_1 hybrids, *Papaver nudicaule* ($n = 7$) $\times$ *P. radicatum* ($n = 35$) and *P. nudicaule* var. *striatocarpum* ($n = 35$) $\times$ *P. nudicaule* ($n = 7$), showed 21_{II}. The latter F_1 was highly fertile, its reduction division normal, and 21 chromosomes passed to each pole. It was explained that out of 21_{II}, those that formed allosyndedically were 7 and those that formed autosyndedically were 14.

Shimotomai's *Chrysanthemums* (1931, 1933) were very peculiar examples; the results obtained by him being

Mating	Meiosis in F_1
Chrysanthemum Decaisneanum (n = 36) × *C. indicum*(n = 18)	27_{II} (autosyndesis)
C. ornatum (n = 36) × *C. indicum* (n = 18)	27_{II} (autosyndesis)
C. japonense (n = 27) × *C. pacificum* (n = 45)	36_{II} (auto- and allosyndesis)

In the parents used, only bivalents were normally observed, and in the F_1 also, owing to auto- or allosyndesis, all the chromosomes were found to be bivalents.

Lilienfeld (1936) interpreted the occurrence, in autohexaploid *Fragaria elatior* (n = 21), of bivalents alone, without any polyvalents, as meaning that, of the 6 genoms, the especially homologous two's were conjugated selectively, resulting in only bivalents and no polyvalents. In my sugar cane 2725 POJ, the appearance of only a small number of polyvalents may also be explained in the same way.

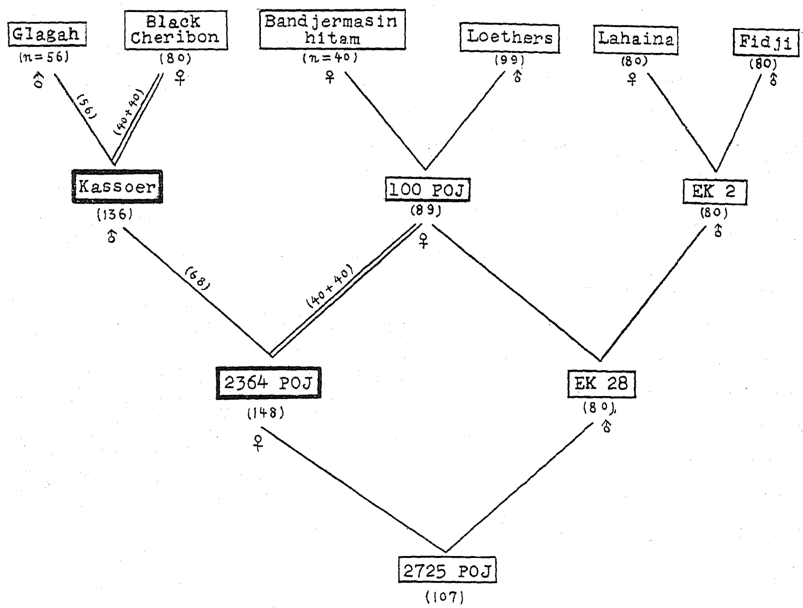


Fig. 38. Pedigree of 2725 POJ (modified from van Deventer 1927). The diploid chromosome numbers in parentheses are shown according to Bremer's counting (1923, 1924, 1928), except in Glagah and Bandjermasin hitam, for which the haploid ones are given. Chromosome doubling occurred in the formations of Kassoer and 2364 POJ.

Since Bremer's work (1923, 1928), it has been known that in the genus *Saccharum*, chromosome doubling occurs on the mother's side when crossed interspecifically. In crossing noble canes with wild canes (he uses the term "nobilitation" for such crossing) and in back-crossing noble canes with the resultant F_1 (his "second nobilitation"), doubling occurred in the maternal chromosome set. But in back-crossing the noble canes with plants produced by

"second nobilitation", namely, in the "third nobilitation", no doubling of the chromosome set took place; and in the crosses that were made in the nobilitations of higher orders, no chromosome doubling could be observed (cf. Fig. 38). It is interesting to note that, by crossing sugar cane produced by third nobilitation, which consequently had no tendency to chromosome doubling, with *Saccharum spontaneum*, doubling of the maternal chromosome set again took place (Bremer 1928). This doubling, therefore, is what occurred for the third time, if counted from the beginning of nobilitation. The doubling of a chromosome set effected by our Cane Breeding Division by crossing the sugar cane with the genus *Sorghum* is a similar example.

No detailed studies are known in regard to the doubling of chromosomes during the courses of fertilization or of zygotic development in the species cross of *Saccharum* or in the genus cross of this genus with others. According to Bremer (1923, 1928, 1929), in crossing *Saccharum officinarum* with *Sacc. spontaneum*, chromosome doubling is ascribed to splitting of the reduced maternal chromosomes, which occurs just at the time of fertilization. In my F_1 of normal type, it is conceivable that the 54 chromosomes contained in the egg cells formed in 2725 POJ, underwent doubling, either during the process of fertilization with the male gamete of sorghum, or in the earlier development of the F_1 zygote.

The morphology of the somatic chromosomes of the genus *Sorghum* was reported by Huskins and Smith (1932) for the first time, the present writer (1936) also having made some observations in this respect on Manchurian Kaoliangs. A pair of peculiarly shaped chromosomes ("A-chromosomes"), having two constrictions and a short segment, were observed in the root-tip cells of these plants. In my materials of F_1 2725 POJ $\times$ *Sorghum vulgare*, these A-chromosomes were in many cases scarecely observable, owing probably to certain conditions of fixing. In both parents, the size and shape of the chromosomes are much alike, making their identification in F_1 plants difficult.

We shall now turn our attention to the relation between the chromosome constitution and the external characters of F_1 plants. The constitution of chromosomes of different types of our F_1 may be seen from Table 4.

Table 4. Chromosome constitution of F_1 plants

Types of F_1	Chromosome constitution
Normal type (sugar cane type)	2C + 1S
Intermediate type	1C + 1S
Dwarf type (terato-type)	1C + 1S

In this Table, C is a haploid set of chromosomes (not genom) of sugar cane, so that in this case, C = 53 or 54, the number found in 2725 POJ. S is a haploid set of sorghum chromosomes, namely, S = 10.

The foregoing relations were already pointed out by Singh (1934 b), but his description of F_1 individuals is inadequate, while nothing is said about meiosis. He made a back-cross of an F_1 individual (2n = 63–64), of no economic value which may be recognized as 1C + 1S, with sorghum. The resultant hybrids showed 2n = 73–74, which may be recognized as 1C + 2S, resembling sorghum most closely in the external characters than any of the F_1 plants that were studied. According to Janaki Ammal (1938), in sugar cane-sorghum hybrids, viable embryos are formed from fertilization of both haploid and diploid eggs. This might have occurred also in my F_1.

As I have discussed in my preliminary note (1938), the normal type of F_1, showing the chromosome constitution 2C + 1S, is more valuable for breeding purposes than the intermediate and dwarf types, each of which being consisted of 1C + 1S. Chromosome doubling in sugar cane is, therefore, important for practical purpose. Some plants of normal type of F_1 showed partial fertility in egg cells, although the pollen grains were abortive, enabling us to obtain several descendants by back-crossing with sugar canes. Studies on the economic value of these descendants are reserved for the future.

Both dwarf and intermediate types showed chromosome numbers corresponding to 1C + 1S, although the morphological differences between the two types and also between individuals of each type are considerable, owing to the unequal genetical contents of the chromosomes involved in each plant. Structural variations in chromosomes were suggested by the fragmentation found, although rarely, in F_1, but the details are unknown.

In closing, I wish to express my sincere thanks to Dr. M. Yamasaki, Chief of our Cane Breeding Division, under whose general directions the studies were carried out. I am also particularly indebted to Prof. F. Kagawa, for having kindly revised this paper and for loaning me valuable literature.

Summary

1) Studies of chromosomes were made in F_1 progenies of the sugar cane variety 2725 POJ crossed with *Sorghum vulgare*.

2) In 2725 POJ, the chromosome number was 2n = 107. Since, in the meiosis of its PMC, univalent chromosomes were observed

together with bivalents, and occasionally multivalents, in the polar view of the heterotypic metaphase, we counted usually more chromosome elements than the haploid number corresponding to its somatic one. The pollen grains were completely sterile. The number of chromosomes of *Sorghum vulgare* was $n = 10$.

3) The F_1 plants were classified by their external characters into three types. The first type resembled sugar cane (normal or sugar cane type), the second was a dwarf (dwarf or terato-type), and the third an intermediate between the above two (intermediate type).

4) The chromosome number of the normal type of F_1 was $2n = 118$. This number arose probably by doubling of the maternal chromosomes, so that its chromosome constitution should be given as 2C + 1S. Here, C represents a haploid set of chromosomes (not genom) of sugar cane and S that of sorghum. Both the dwarf and the intermediate types of F_1 showed $2n = 64$, their chromosome constitutions being regarded as 1C + 1S. As the result of genetic inequalities, the two last-named types exhibited considerable morphological differences between them and also between different individuals of a type.

5) In the normal type of F_1, about 10 univalents were found in meiosis, which may be recognized to have come from sorghum.

6) In the intermediate type of F_1, usually 37 chromosome elements, including uni-, bi-, and sometimes multivalents, were counted in its meiosis. Of these, the number of univalents exceeded 10, which may be owing to the fact that many chromosomes of the haploid set of sugar cane conjugated autosyndedically to form bi- or multivalents, and the sorghum chromosomes remained univalents. Whence, it may be concluded that 2725 POJ is, at least partially, of an autopolyploid nature.

7) The doubling of chromosomes in the F_1 of normal type, resulting in chromosome constitution 2C + 1S, is probably the result of splitting of the maternal chromosomes, as pointed out by Bremer (1923, 1928).

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Spermatogenesis of the Crayfish, *Cambarus virilis*, with Special Reference to the Golgi Material and Mitochondria[1)]

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Introduction

The peculiar non-flagellate or vesiculate spermatozoa of the Decapod Crustacea and the manner of their development have been the subject of numerous investigations. Despite this fact, Decapod spermatogenesis still presents puzzling phases. This is due in part to the confusion which has arisen from the extreme variety in the form and appearance of the sperm within the group and in part to the relative scarcity of information concerning the cytoplasmic components which are known to play an important role in the development of the more typical sperm of other groups.

Renewed impetus toward the study of Decapod sperm has been derived from a review by Bowen ('25) who, without an opportunity to study the development of non-flagellate sperm, attempted to correlate the various structures described in the spermatogenesis of several typical forms, notably *Ascaris* and the Decapods, with those found in flagellated sperm. He suggested that the "glass body" or "refringent body" of the *Ascaris* sperm and the "Schwanzkapsel" or "vesicle" of the lobster and crayfish sperm might represent the

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1) This paper has had to be printed without the author's corrections, for, despite the fact that we have twice sent the proof to him and also have tried to communicate with him through the authorities of the State University of Iowa and Emory Junior College in Valdosta, we have received nothing from him even after the long delay which this has occasioned. Editor.

acrosome. Sturdivant ('34) has shown that this suggestion is correct for *Ascaris megalocephala.* Grabowska ('29) working on the European crayfish, *Potamobius astacus,* described the behavior of the cytoplasmic inclusions during spermatogenesis but came to no definite conclusions concerning the existence of an acrosome. Nath ('32) describes the acrosome of the sperm of *Paratelphusa spinigera,* a fresh water crab, as a circular band fused with the margin of a nuclear cup inside which lies a vesicle of mitochondrial origin. In 1937 Nath described the spermatozoon of *Palaemon lamarrei,* a prawn, in substantially the same terms with the exception that the Golgi material and the mitochondria are both considered to liquefy to form the contents of the vesicle. Further, "the acrosome is conspicuous by its absence". Since Fasten's ('14) study on *Cambarus*

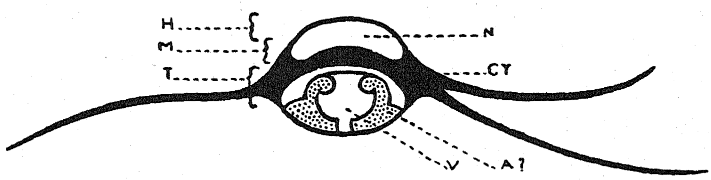


Diagram 1. Diagrammatic illustration of the sperm of the European crayfish, *Potamobius astacus,* (based largely on Hermann). The terms to the left of the figure follow the regional terminology of Koltzoff and those to the right of the figure are derived, with slight modification, from Bowen. A, 'capsule' (refringent material or acrosome); Cy., cytoplasm; H, head; M, midpiece; N, nucleus; T, 'Schwanzkapsel' (tail piece or tail capsule); V, vesicle.

virilis and *Cambarus immunis* was made without the aid of the modern techniques for demonstrating cytoplasmic structures, a re-examination of the crayfish sperm seemed especially desirable. Accordingly it is the purpose of this paper to present the results of a study on spermatogenesis of the crayfish with the use of modern cytological techniques and to discuss the relationship of the various structures observed to those described during the genesis of typical sperm.

I am indebted to Professor H. P. Sturdivant for suggesting the problem. It was begun at Emory University under his direction, continued under Doctor W. B. Baker, and completed at the State University of Iowa under Professor H. W. Beams. I should like to express my gratitude to all three for their advice and encouragement. In addition, I am indebted to Professor J. H. Bodine for the facilities of the University and Iowa Lakeside Laboratories, as well as to Professor L. O. Nolf, Associate Director of the Lakeside Laboratory. I am deeply appreciative of the assistance of Miss Bernette Bohen whose drawings illustrate this paper.

Material and Methods

Crayfish of the species *Cambarus virilis* were obtained from Turkey Creek near Iowa City, Iowa, from Lake Okoboji (Little Miller's Bay), and from the pools of the Fisheries Station at Freeport, Iowa.

Whenever possible, the three-lobed testis was dissected out immediately after the capture of the animal and fixed. Sperm from the vasa deferentia and from the seminal receptacle of the female were also examined in smear preparations both vitally and after fixation and staining.

Testicular proliferation is never entirely absent, although there is a definite period of great activity beginning in the late spring and continuing until late in September. In October and November copulation occurs, and eggs apparently are laid extremely early in the spring.

Material was fixed for general purposes in Bouin's, Carnoy's, Zenker's, Helly's, Maximow's, Flemming's, Hermann's, and Champy's fluids. Sublimate-acetic, sublimate, and the Carnoy-Lebrun solutions were also used.

Mitochondrial preparations were fixed in Regaud's, Flemming's without acetic, Benda's (modified Flemming's), Champy's, Hermann's without acetic, and several other fluids.

The Golgi material was demonstrated with osmic acid after fixation with Champy's fluid and the Mann-Kopsch-Weigl solution, or with silver nitrate after fixation with Cajal's and Da Fano's fluids.

Some material was run through the ordinary methods for paraffin infiltration, but the Peterfi double infiltration technique gave best results. The most useful stains proved to be Heidenhain's hematoxylin after iron alum mordanting, the Feulgen "Nukleal Reaktion" (fuchsin-sulphurous acid) counterstained in light green, the Benda alizarin-crystal violet method, the Regaud hematoxylin method, the Flemming triple stain, and safranin and light green.

Observations

Examination of the testis in early June shows it to be packed solidly with spermatogonia with a few acini showing later stages. Even at this time, the vasa deferentia contain considerable numbers of spermatozoa. During July and August, the proportion of later stages rises steadily, and just before copulation the testis contains few spermatogonia-filled acini and is largely filled with late spermatids and nearly mature sperm. The vasa deferentia are packed solidly with apparently mature sperm.

Spermatogonia and spermatocytes

1. Spermatogonia

Regaud preparations of spermatogonia (fig. 1) in the resting condition reveal the presence in the large, almost oval nucleus of one to several massive karyosomes which lie at some distance from the nuclear wall. A few chromatin granules lying against the wall represent the only other stainable nuclear materials. Frequently osmic preparations will demonstrate the karyosomes.

At this stage the rather sparse cytoplasm contains large masses of mitochondria-like material. After Regaud-hematoxylin, these masses stain very heavily but radical destaining reveals that each mass is composed of separate filaments which are frequently irregular. Champy preparations reveal these filaments with greater distinctness and less apparent distortion. The Benda and Flemming triple stains demonstrate much the same type of structure although numerous granules take crystal violet in the first named stain in such a fashion as to obscure the picture somewhat. These granules apparently represent the structures identified as mitochondria in supravital preparations by Grabowska ('29).

Also, the mitochondrial masses of my description probably represent the chromatoid masses figured by Fasten ('14) in the spermatogonium. Supravital preparations with Janus green B do not seem to demonstrate the mitochondria of these cells with any greater precision.

Osmic preparations (fig. 17) reveal a few scattered dictyosomes none of which lie particularly near the nuclear membrane. Random granules in the nucleus and the karyosomes frequently darken with osmic acid and silver nitrate but fall far short of the intense blackening of the Golgi material.

Dividing spermatogonia (fig. 2) fixed in Champy and stained with hematoxylin, show the mitochondria as separate, definite filaments lying at the end of the wide spindle and, to a lesser degree, in the cytoplasm surrounding it. No filamentous mitochondria were observed between the spindle fibers as Reinhard ('13) has described them for *Potamobius*. In these preparations, the presence of an unusually large number of chromosomes is most obvious. The centrosome, however, is an extremely inconspicuous structure and is frequently not to be observed.

2. Primary spermatocytes

Very early spermatocytes show the mitochondria as reassembled masses much as they have been described for spermatogonia (fig. 3).

The nucleus has now lost its karyosomes (fig. 3), and appears almost amorphous in the Regaud-fixed hematoxylin preparations on which the immediately foregoing account of the mitochondria is based. Only a few lightly stained strands marked the development of prophase chromosomes. In later stages chromosome organization is more obvious (fig. 4), but formalin bichromate-fixed chromatin continues to manifest a lack of affinity for hematoxylin.

During the late prophase, one or two chromatoid bodies can be demonstrated in the cytoplasm after most fixatives. These small rounded bodies are rendered most conspicuous after Flemming fixation by the safranin of the Flemming triple stain or by crystal violet as in the Benda method. Champy-fixed preparations are equally suitable and Carnoy or Carnoy-Lebrun will also suffice.

Champy-fixed post osmicated spermatocytes (fig. 18) show the Golgi material collected in an idiosomal area during the early prophase but scattered more or less evenly around the nucleus in later stages (fig. 19). Here the typical form of the individual dictyosomes is more obvious than in the previous spermatogonial stage. Each Golgi body possesses an osmiophilic rim and a lightly impregnated central portion. Frequently such preparations show an apparent contraction of the nucleus leaving it surrounded by a halo-like clear area although the chromatin material appears to be well fixed.

Neutral red stained vital preparations show a system of tiny vacuoles in contrast to the large units of the vacuome figured by Grabowska ('29) at this stage. These small vacuoles never appeared to be congregated in an idiosomal area even in very early spermatocytes.

As the nuclear wall disappears and the chromosomes range themselves on the spindle for the first spermatocytic division, their dumbbell shape is very evident. In polar views of the metaphase it is possible to distinguish approximately 100 chromosomes. This is the number described by Fasten ('14) and is probably the correct one for *Cambarus virilis*.

In Champy-fixed preparations of dividing primary spermatocytes, which have been stained with Heidenhain's hematoxylin, there appear numerous finely filamentous mitochondria which lie outside the area of the spindle and show a slight tendency toward a polar concentration (fig. 5). Frequently such filaments appear to end in knobs.

Janus green B vital preparations present a strikingly similar appearance.

Clearly visible at this stage are two chromatoid bodies which frequently lie beyond the centrosome at or near the poles of the cell (fig. 5). The tremendous size of crayfish primary spermatocytes

makes the occurrence of both chromatoid bodies in the same plane of section somewhat infrequent. It is therefore difficult to trace them, but it is obvious that at times the two chromatoid bodies pass to the same pole as Fasten ('14) has said. The chromatoid bodies are very nearly as well demonstrated by hematoxylin after Bouin's or Carnoy's solutions as they are after Champy's and Flemming's. Occasionally they blacken lightly in osmic acid and silver nitrate, but they do not appear to be chemically similar to the Golgi material as suggested by Nath ('32 and '37).

3. Secondary spermatocytes

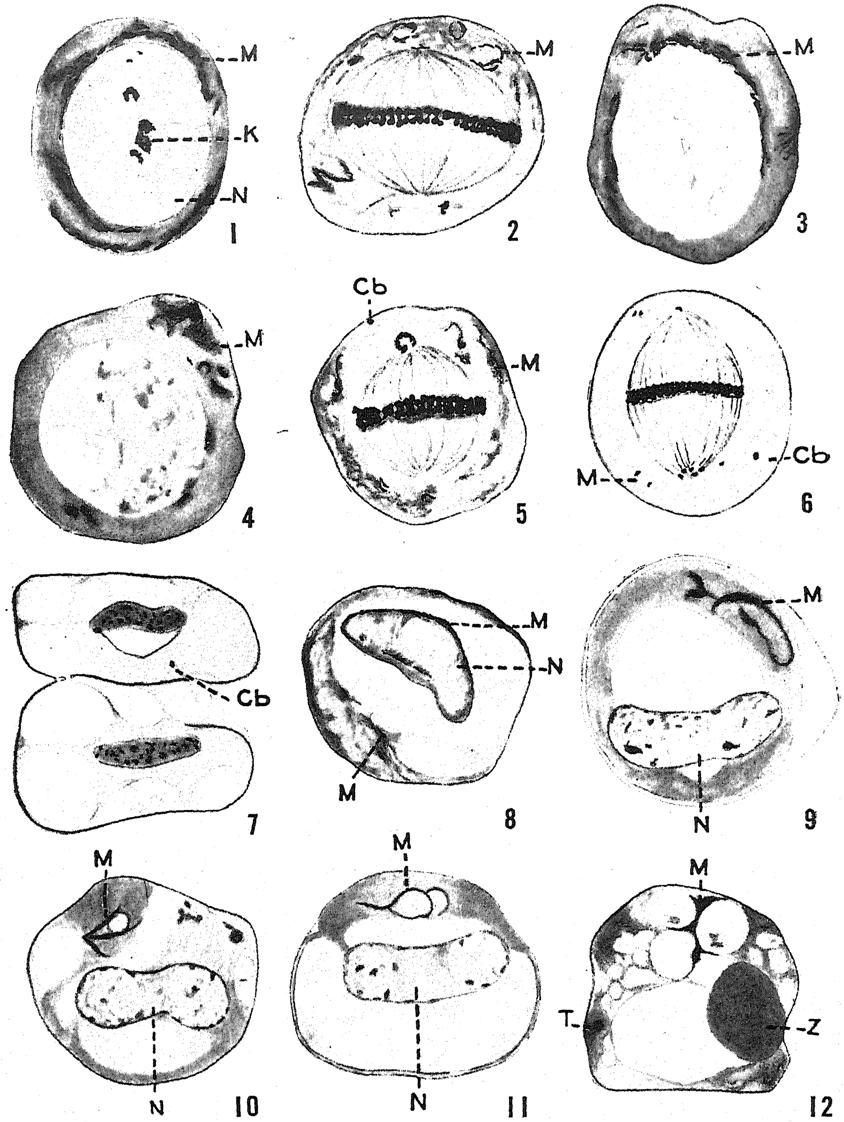
The second spermatocytic division follows closely upon the first.

The chromosomes of a dividing secondary spermatocyte (fig. 6) appear to be about half the size of a chromosome of the first spermatocytic metaphase and are dumb bell shaped as before.

Champy-hematoxylin preparations show irregular granular bodies (fig. 6) which closely resemble the knob-like termini of the mitochondria of primary spermatocytes. Difficulties in preparation which have been developing since the onset of maturation become acute here, for the cytoplasm is so filled with watery vacuoles in the living condition that it is not surprising that fixation is a major difficulty and likely to result in disappointing preparations. However, quick dissection and fixing of the tissue is somewhat helpful. In telophases of the second spermatocytic division (fig. 7), Flemming, Champy and Regaud preparations do not reveal definitive mitochondria. Nevertheless, Janus green B shows tiny, irregular, rod-like mitochondria lying close to the nuclear wall and surrounding each of the daughter nuclei. Mitochondria could not be identified by Grabowska ('29) after the onset of maturation, and Fasten ('14) neither describes nor figures mitochondria during the second spermatocytic division, although the methods which he favors for the demonstration of chromatoid bodies are essentially mitochondria methods.

A chromatoid body is usually to be observed lying slightly to one side of the spindle in secondary spermatocyte divisions. Around it is manifest a clear area as seen before in primary spermatocytes. Occasionally a chromatoid body occurs at each pole, and Fasten ('14) shows photographs of both chromatoid bodies passing to the same pole. This latter condition was not observed in my material.

The Golgi material again appears around the second maturation spindle as tiny batonettes scattered through the cytoplasm. The comment made above on the difficulty of securing adequate cytoplasmic fixation in this stage applies equally well here.



In figures 1–12 as well as in all other figures 13–48, the following denotations are used: A., acrosome; Ab., acroblastic mass; C., centrosome; Cb., chromatoid body; G., Golgi material; I., idiosome; K., karyosome; M., mitochondria; N., nucleus; Nk., nebenkern; T., tigelle (distal centriole?); V., vesicle or 'Schwanzkapsel'; V^1., primary or major subdivision of the vesicle; V^2., secondary or apical subdivision of the vesicle; Z., cytoplasmic (acroblastic?) mass.

Figs. 1–12. 1. Resting spermatogonium, Regaud preparation. 2. Dividing spermatogonium, Champy fixation, hematoxylin. 3 and 4. Resting primary spermatocytes, Regaud preparation. 5. Dividing primary spermatocyte, Champy fixation, hematoxylin. 6. Dividing secondary spermatocyte, Champy fixation, hematoxylin. 7. Telophase of second spermatocytic division, Hermann fixation, Flemming triple stain. 8. Early spermatid, Regaud preparation. 9, 10, 11 and 12. Spermatids during capsule formation, Regaud preparation.

Spermatids and Spermioteleosis

1. Early spermatids

The early spermatids of *Cambarus virilis* (fig. 40) contain a flattened plate-like nucleus, which appears somewhat crescentic in side view, away from which the cytoplasm has nearly always retracted to leave large clear areas traversed by cytoplasmic strands (fig. 8). Adequate chromatin fixation as with Bouin's (fig. 40) shows the nucleus to contain scattered chromatin granules.

The presence of filamentous mitochondria lying against the nuclear membrane is demonstrable in Regaud preparations but by no means all the mitochondria are found there. Scattered through the non-vacuolated portion of the cytoplasm and lying against the cell wall are numerous finely filamentous mitochondria (fig. 8). Nath ('32) has described the mitochondria as vesicular in this stage.

The chromatoid body is relatively unchanged in these early spermatids (fig. 40) but soon moves to the periphery of the cell and can no longer be followed with certainty. Fasten ('14) thinks that it passes out and has no further relationship to sperm formation. Grabowska ('29) describes a small rod-like body lying in relatively the same position as that of the chromatoid body depicted in fig. 40 as disappearing from later spermatids. She is inclined to believe that this structure represents the chromatoid body in *Potamobius*.

Directly above the nucleus lies the centrosome (fig. 40). It is a compound body consisting of a discrete central granule surrounded by a number of smaller granules.

The Golgi material of early spermatids is unidentifiable in osmic acid and silver nitrate preparations. Mixtures of crystal violet and neutral red do no demonstrate definite structures in living spermatids. Finely granular material in the general cytoplasm stains lightly with crystal violet. In later stages (fig. 20) osmic acid is reduced by similar granular material lying around and within the cavity of the doughnut-shaped nucleus.

Nath ('32) has described the Golgi material in this stage as consisting of a number of large dictyosome-like bodies which have fused or are fusing to form an acrosome. In similar preparations (hematoxylin after Flemming fixation) (fig. 8) no such structures are visible in my material.

2. Behavior of the nucleus

Feulgen's "Nukleal-Reaktion" makes it possible to follow nuclear changes with ease throughout spermatogenesis. Figure 33 shows a

primary spermatocyte in such a preparation. This figure is here included as evidence of the character of the reaction.

The nucleus of the spermatid, as followed in Feulgen preparations counterstained with light green, undergoes rather drastic changes as spermioteleosis continues. The flat plate-like nucleus described above begins to thin out in the center and finally develops a central opening (fig. 34). A side view of a section of a slightly later stage (fig. 35) shows the nucleus to be definitely doughnut-shaped with an obvious central opening. In osmic acid preparations (fig. 20) the same appearance is somewhat enhanced by the blackening of protoplasmic strands which pass through the central space. The nucleus condenses somewhat (fig. 36) but continues to be defintely a thick ring, the center of which is filled with cytoplasmic material.

As the capsule is formed, it shows in Feulgen preparations (fig. 37) as a clear hemispherical vesicle around the base of which the nucleus, now a laterally flattened ring, fits closely. Figure 37 shows such a spermatid cut slightly obliquely. Figure 40 shows the nucleus as seen in an earlier stage as viewed from above and slightly to one side. This figure is taken from a preparation stained with the Flemming triple stain in which the situation is almost as clear as in Feulgen preparations. In the late spermatid (figs. 13, 14 and 30), the nucleus is not stained by hematoxylin after Regaud or by crystal violet as used in the Benda method. Long-method Heidenhain's preparations produce such a blackening of the vesicle and the cytoplasmic material below the nucleus that it is not surprising that the nucleus has been assumed by many early workers to be in the vesicle, and by others to be the cytoplasmic cap underneath. (Koltzoff '06 and others).

As the "cytoplasmic arms" begin to spin out, the Feulgen reaction reveals them to be principally of nuclear origin (fig. 39). Andrews ('04) suggested this long ago but all the evidence has been preponderantly against his interpretation. It has generally been considered (Koltzoff '06, Fasten '14, '18, '24, '26, and others) that the arms are of mitochondrial origin. Other investigators have described the nuclear-mitochondrial cup as the probable source of the arms. Binford ('13) was unable to determine whether the nucleus or the mitochondrial ring lying above it gave rise to the arms of the sperm in the crab *Menippe*.

The mature sperm retains the nucleus in the above described position. Spermatozoa (fig. 38) exploded by sudden pressure and fixed immediately show the nucleus as a flattened ring projecting into the arms which are here not normal in extent. Figure 32 shows a similar preparation stained in iron-hematoxylin. The ampulla-like appearance of the bases of the arms here is probably nearer normal.

Sperm fixed without crushing do not differ from late spermatids (fig. 39) in the extent of the nucleus.

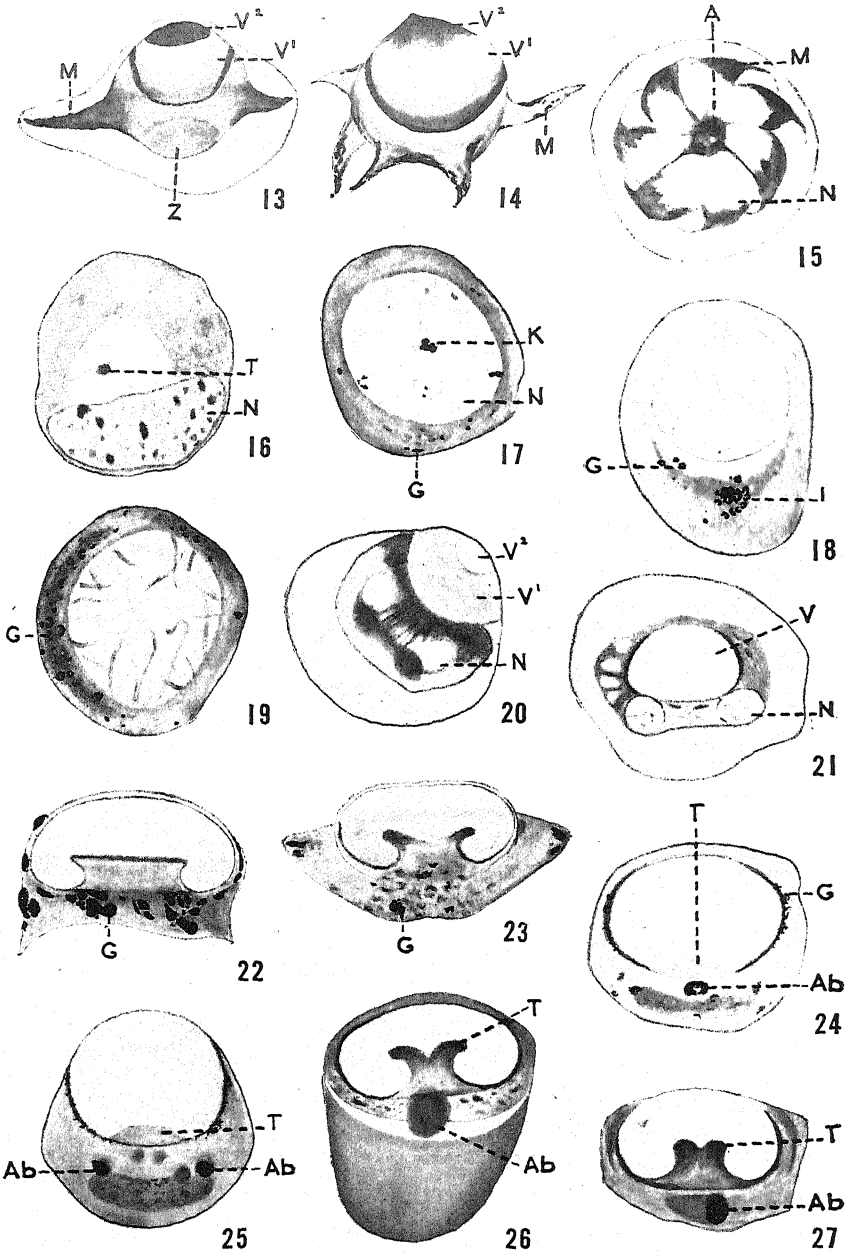
Examination of the lobster sperm reveals a very similar situation. The nucleus appears to be a thin ring lying at the base of the cylindrical capsule. No extension into the arms was noted.

3. Cytoplasmic structures during later spermioteleosis

Shortly after the nucleus begins its transformation, the mitochondria undergo a process of aggregation to form heavy filaments or rods (figs. 9 and 10). These filaments are associated almost from the beginning with cytoplasmic vesicles (fig. 10) which increase in number and begin to fuse (fig. 12). This process continues and results in the formation of the tail-capsule or vesicle. Nath ('34) has interpreted the vesicles themselves as mitochondria while Binford ('13) describes vesicles as arising in the general cytoplasm and fusing to form one large vesicle which becomes the capsule. Fasten ('14) says, "A vesicle appears". Grobben ('78), Koltzoff ('06) and others derive the vesicle from formed bodies, "chitinartige Anlage", which fuse. Grabowska ('29) favors the same explanation but derives the "chitinartige" material from the vacuome (Internum of the Golgi apparatus). In Hermann-fixed preparations stained with the Flemming triple stain, the mitochondria behave in no wise differently at comparable stages but the vesicles are not so obvious. Figures 43, 46, 47 and 48 show the appearance of the developing capsule. The peculiar cytoplasmic structure in figures 46 and 47 appears to be of mitochondrial origin. Other mitochondrial preparations present the same appearance. It seems, therefore, that something comparable to the nebenkern organ of flagellate sperm is here formed and that it gives rise to the capsular wall. Even if one assumes a complete passivity of the mitochondria during this process it is obvious that they are incorporated in the developing wall. The upper part of the capsule wall (figs. 13, 14, 28, and 48) is demonstrated with the greatest definiteness in mitochondrial preparations.

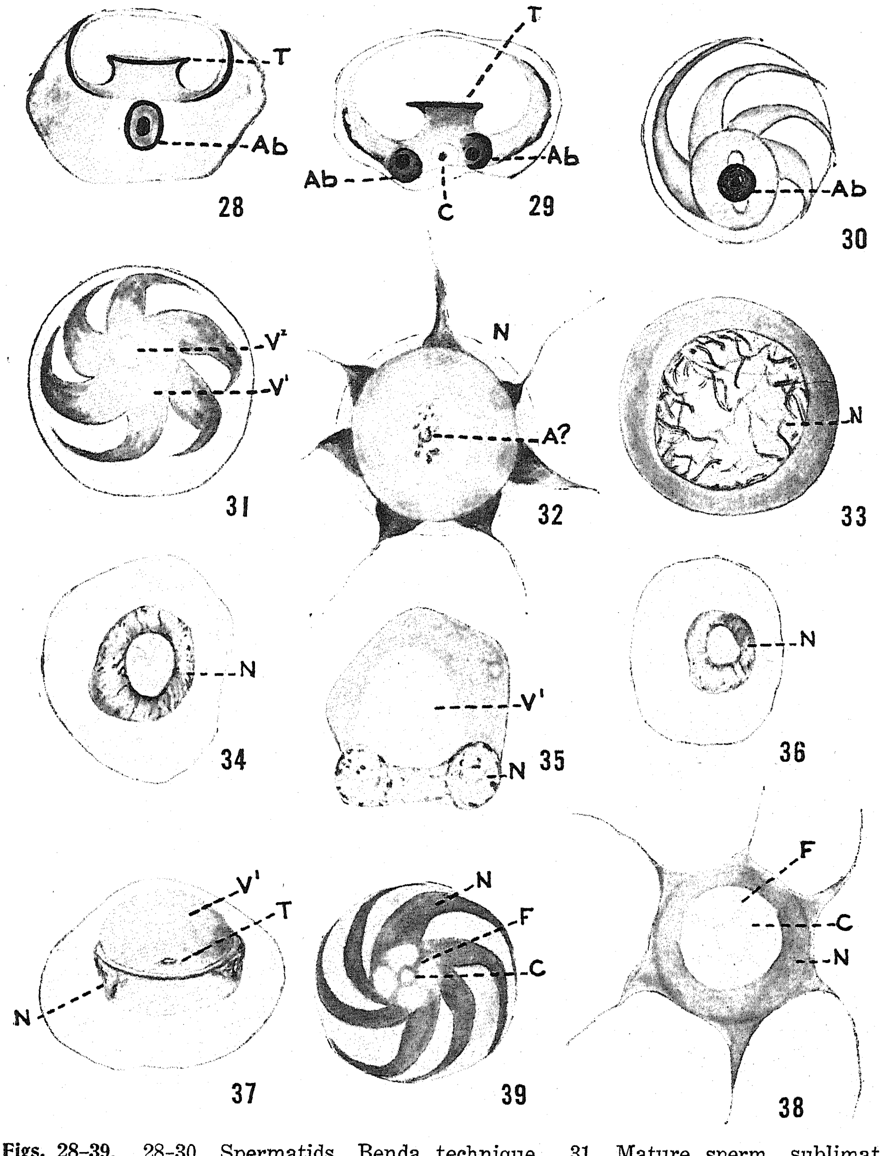
Some of the mitochondria are carried out in the cytoplasmic covering of the nuclear arms where they remain demonstrable in late spermatids (figs. 13 and 14) and even in mature sperm (fig. 15).

The centrosome now moves into the center of the nuclear ring and hollows out into a ring from which fibers radiate into the arms (fig. 39). In favorable preparations of mature sperm (fig. 38) one can see that there is a centrosomal fiber for each arm. Fasten ('14) describes the same structure with the exception that he does not consider the centrosome to form a ring. Counterstains after Feulgen preparations demonstrate this structure beautifully.



Figs. 13–27. 13 and 14. Late spermatids, Regaud preparation. 15. Mature sperm, Champy fixation, hematoxylin. 16. Spermatid, Hermann fixation, Flemming triple stain. 17. Spermatogonium, Champy fixation, osmic acid impregnation. 18. Early primary spermatocyte, Champy fixation, osmic acid impregnation. 19. Later primary spermatocyte, Champy fixation, osmic acid impregnation. 20, 21, 22, 23, 24, and 25. Spermatids, Champy fixation, osmic acid impregnation. 26 and 27. Spermatids, Flemming triple stain.

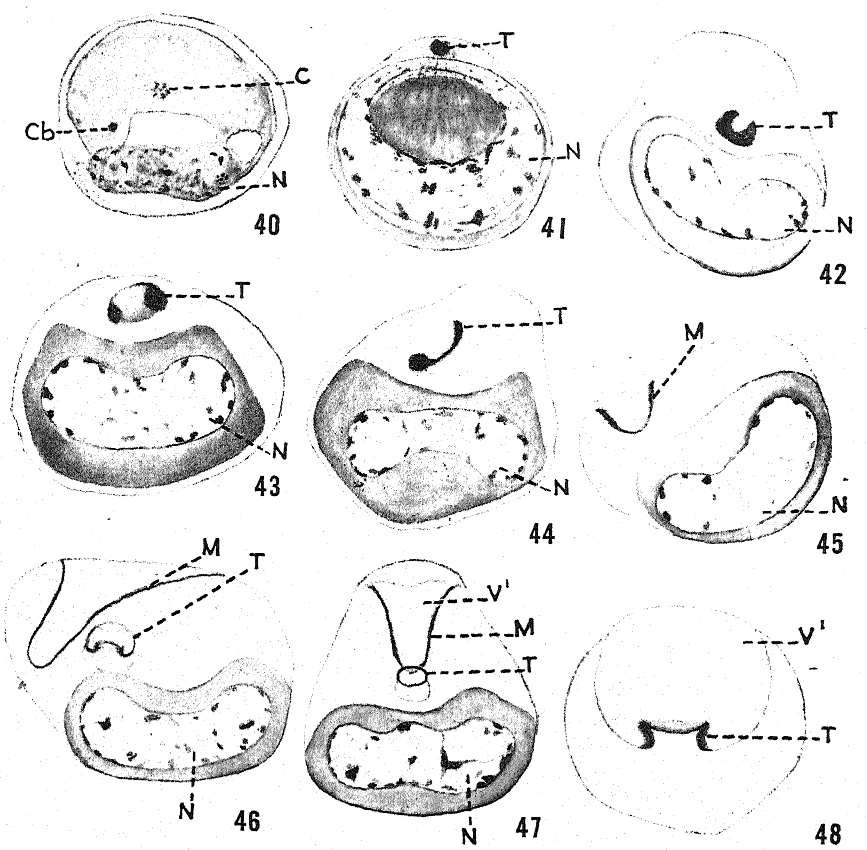
Near the developing capsule there appears a peculiar formation which is startlingly like the chromatoid body, at least during the early stages of its visibility (figs. 12 and 16). The connection be-



Figs. 28–39. 28-30. Spermatids, Benda technique. 31. Mature sperm, sublimate fixation, hexatoxylin. 32. Mature sperm crushed by pressure on cover slip, Champy fixation, hematoxylin. 33. Primary spermatocyte, Ludford fixation, Feulgen method counterstained with light green. 34, 35, 36, and 37. Spermatids, Ludford fixative, Feulgen and light green. 38. Mature sperm crushed by pressure on cover slip, Ludford fixative, Feulgen and light green stain. 39. Spermatid, Ludford fixative, Feulgen and light green stain.

tween the chromatoid body and this new structure is rendered problematic by the impossibility of tracing the chromatoid body after the early spermatid stage and by the peculiar behavior of the new body as well as by its constant presence in the cell. Chromatoid bodies apparently are not apportioned equally to the spermatids (Fasten '14, '18, '21, '24, '26 and '36). In my material, structures resembling the chromatoid body are to be found in the sloughed cytoplasm surrounding late spermatids. (Wilson '13, Bowen '24, Fasten '18 and '26, Grabowska '29).

This body of uncertain origin (tigelle) now pulls out into a thick-armed "U"-shaped structure (fig. 42). As this stretching continues it is obvious that the walls are growing around and down to form a cup (fig. 43). A view of this developing cup from the side and slightly above is shown in figure 44. Presently the completed cup comes to be open at both ends and begins to show a double nature,



Figs. 40-48. Spermatids during vesicle wall formation, Hermann fixation, Flemming triple stain.

the meaning of which is not quite clear. It appears as if a double cup were developed and that the two parts then telescope into each other or that the upper or heavily staining portion grows down and around the lower portion. In the Flemming triple stain, the upper portion has a strong affinity for safranin while the lower part becomes an indefinite gray. While these changes have been taking place, the mitochondrial wall of the capsule, which lies nearby, has progressed considerably toward completion and the now barrel-shaped body approaches it closely (fig. 47). It then pushes up into the capsule (fig. 37) causing the invagination of the lower floor of the capsule. A patent opening in the capsule wall coincides with the lumen of the cylinder as the body being described may now be called with accuracy. The walls of the two structures now fuse (fig. 48) and the inner opening into the capsule is enlarged by the outgrowth of its ring-like margin (figs. 26 and 27).

This produces the recurving of the walls of the invaginated portion described as causing the capsule to assume the appearance of "an elliptical bowl, inverted and with thick walls that are invaginated at the mouth and somewhat turned back into the cavity" (Andrews '04).

Grabowska ('29) describes a structure in the *Potamobius* sperm which moves up to the base of the vesicle and there disappears just as the very slightly invaginated portion of the "bowl" of the sperm makes its appearance. It is her opinion that this body is the "tigelle" of the early French workers on Decapod sperm.

As Grabowska pointed out, the "tigelle" does not reduce osmic acid and has no apparent relationship to the Golgi material. It can be demonstrated side by side with the mitochondria and sharply differentiated from them by its staining reaction. Therefore it is likely that this body is of centrosomal origin. Labbe ('04), Gilson ('87) and others have identified such a structure as centrosomal. The term "tigelle" has come to mean "a central body", probably derived from the centrosome and is applied in that sense here.

The Golgi material becomes readily demonstrable with osmic acid in later spermatids. Figure 24 shows its appearance as the capsule is in the earlier stages of development. The Golgi material lies in the general mass of cytoplasm below the nucleus and around the walls of the lower two-thirds of the vesicle (fig. 22). While there is apparently some inconstancy in the time at which it occurs, there is soon visible a tendency toward aggregation of the separate dictyosomes (figs. 22 and 23). These groups unite finally into one or two acroblastic masses (figs. 24 and 25). Figure 27 shows the same phenomenon in Flemming-stained material and indicates the begin-

ning of acrosome formation. Here the heavily staining mass is lightly osmicated Golgi material restained with crystal violet, while the lighter adjacent area represents acrosomal material. Figure 26 shows a similar preparation in which no Golgi material is visible. As the sperm nears maturity, one frequently sees structures similar to those shown in figures 29 and 30 in which the acrosome whether present as one or two bodies is still double in nature. These figures are drawn from Benda preparations but Champy-osmic preparations radically bleached show the same structure. From this time forward, even in the sperm from the lower part of the vas deferens of mature males, it is possible to demonstrate the acrosome equally well with osmic acid or with mitochondrial techniques. Figure 15 is from a Champy-hematoxylin smear. It is apparent in most of these preparations that the acrosome is essentially bipartite and includes some Golgi material, at least until very late stages. Grabowska ('29) describes a Golgi derivative in the mature sperm which closely resembles the acrosome as described here. She is of the opinion, however, that it is only one of the Golgi derivatives in the sperm and she does not elect to interpret either as the acrosome.

In her material, the body in question has an osmiophilic rim and an osmiophobic center. The osmiophobic portion (Internum) consists of the assembled vacuome. She was able to demonstrate this structure in neutral red.

In my material, a small body lying in the bottom of the sperm and generally to one side stains with neutral red and Nile blue sulfate. A system of vacuoles and the extracellular material which appears to glue the spirally coiled arms of the sperm to the capsule also stain. The largest body of this group appears definitely vacuolar and may represent the droplet described by Andrews ('04) and Fasten ('12).

If the sperm be flattened by pressure the acrosome appears to fragment as one sees it in figure 32.

The Golgi material does not appear to bear more than a topographical relationship to the formation of the vesicle (figs. 22 and 24). One sees no indication of secretory activity as the vesicle increases in size.

Figures 20 and 25 show a small "secondary vesicle", which is previously undescribed for the crayfish sperm. Its extreme lightness in osmic preparations makes it unlikely that it is of mitochondrial origin although it goes intensely black in Regaud preparations (figs. 13 and 14). In sublimate hematoxylin preparations it appears as a clear vesicle (fig. 31) with a fairly definite rim. Binford ('13) and Nath ('32) describe a similar structure as being

derived from the centriole in certain Brachyura. Fasten ('18) says that it develops quite independently in *Cancer magister.* The secondary vesicle appears to bear no relationship to the centriole in *Cambarus virilis* nor is there any evidence that it is bounded by a "chromatin ring" as described by Fasten ('18) for the crab.

Discussion

The primary point of modern interest in the spermatozoa of the higher Crustacea has been the question of the validity of Bowen's suggestion that the vesicle or tail-capsule of these sperm represents the acrosome. The studies of Grabowska ('29) on *Potomobius astacus,* of Nath ('32 and '37) on *Paratelphusa spinigera* and *Palaemon lamarrei,* and this present work on *Cambarus virilis* have all sought to answer this question.

Nath has come to the conclusion that in the Brachyura the vesicle arises from the fusion of vesicular *mitochondria.* In *Palaemon,* a macruran, he believes the vesicle to be produced by the general liquefaction of the cytoplasm including the Golgi material and the mitochondria. Grabowska describes a possible relationship between the Golgi material and the vesicle which transcends anything implied in Bowen's statement and further qualifies her answer by pointing out that, "Möglicherweise verdankt die chitinartige Anlage der Schwanzkapsel ihre Genese dem granulierten und Mitochondrienreichen Protoplasma, in welchem ihre Anlage zuerst auftritt; sie ist als ein ergastoplasmatisches Gebilde aufzufassen und der mit dem Mitochondrienkörper der Spermatiden flagellifera zu vergleichen, der auch eine ergastoplasmatischer Komponente enthält. Dieses Problem kann ich leider nicht positiv lösen und somit auch die Frage nach der Existenz eines Acrosoms in Spermium bei *Potamobius astacus* nicht beantworten." In my material the vesicle does not appear to develop through the agency of the Golgi material but is found instead to develop in close association with the mitochondria.

Therefore, in the light of these findings, it would appear that the vesicle of the Decapod sperm cannot be considered to be an acrosome but is, on the contrary, very probably of mitochondrial origin.

Another point of interest arises from the different interpretations of the mitochondrial role in vesicle formation. If what Binford ('13) and Fasten ('18) describe as mitochondria are in reality mitochondria, then the structure which Nath ('32) has described in *Paratelphusa* as growing over the surface of the vesicular wall is in all probability a nebenkern organ. In other words, the essentially mitochondrial techniques used by Nath have enabled him to carry

our knowledge of vesicle wall formation in the Brachyura a step further. This interpretation appears to be consonant with the facts as stated and is in keeping with my own findings and the tentative suggestion made by Grabowska ('29) in the foregoing quotation.

The behavior of the structures described by Nath as vesicular mitochondria is so reminiscent of the capsule-anlage as described by Grobben ('78), Koltzoff ('06) and others as to suggest the probable identity of the two. If such is the case, these are the structures which Bowen suggested were really the Golgi bodies. They have been described side by side with the mitochondria by Grobben ('78), Koltzoff ('06), Retzius ('09), and Reinhard ('13). Fasten ('14), Binford ('13), Grabowska ('29) and the present work identify structures other than the capsule-anlage as mitochondria. Nath is alone in interpreting them as mitochondria.

The evidence against the Golgi nature of these capsule-anlage, i.e., against Bowen's hypothesis, need not here be restated except to point out that Nath, Grabowska, and I have been unable to discover an association between the Golgi material and the capsule-anlage which is in any way similar to the findings of Sturdivant ('34) in *Ascaris*, Payne ('27) in *Gelastocoris*, Veinov ('25) in *Gryllotalpa*, or Papanicolau and Stockard ('18) and Gatenby and Woodger ('21) in the guinea pig.

The case made out for the acrosomal nature of the body described as such in this paper must rest upon the fact that it appears to be the only acrosome-like aggregation of Golgi material and, or, its transformation product so far described from osmic preparations in the Decapod crustacean sperm. Grabowska's description of the constant appearance of such a body in *Potomobius* is in essential agreement with my own findings. Nath's description of the acrosome in *Paratelphusa*, while it cannot be discredited, can at least be impeached on the basis of the technique used and on considerations already advanced.

It is apparent from the descriptions of the early behavior of the nucleus in *Pagurus* (Koltzoff '06), in *Menippe* (Binford '13), and in *Paratelphusa* and *Palaemon* (Nath '32 and '37) that there is a distinct possibility that the nucleus in these forms becomes ring-like with a central opening as it has been shown to do in *Cambarus*. This creates the presumption that the Decapod sperm is not necessarily "just like a flagellate sperm". This, at least, is definitely true of the spermatozoan of *Cambarus virilis* which, while it can be interpreted by supposing an even greater fore-shortening than heretofore demanded by those who seek to describe exact positional homologies between the flagellate and the vesiculate sperm (Koltzoff '06, Nath

'34), is much more logically described, as it was originally, as a radially symmetrical or very nearly radially symmetrical structure. This interpretation does no violence to the part-for-part homologies which naturally exist and is in better keeping with the facts that a ring-like nucleus from which radial arms arise is located in the morphological midsection of the sperm, that the proximal(?) centriole is ring-like with radiating fibers, that the "tigelle" (distal centriole?) is a ring albeit somewhat flattened, and that the vesicle is circular in cross-section. Also the acrosome is centrally placed in the lower portion of the sperm (fig. 15).

Finally Terni ('37) using micro-dissection technique on the lobster sperm, showed that pressure causes an eversion of the capsule, not in the manner of Koltzoff as Terni stated it, but rather in the fashion described by Binford ('13). That is to say, eversion of the capsule did not force the "nucleus" down but appeared to retract it into the central tubule of the capsule. This, together with the presence in a typical Decapod sperm of the nucleus at the base of the capsule, of a central tubule or central space in the primary vesicle, and of a "secondary vesicle" in the upper part of the primary vesicle, suggests the possible general application of the mechanism of fertilization described by Binford ('13).

Summary and Conclusion

1. The Golgi material exists in the spermatogonia and spermatocytes of *Cambarus virilis* in the form of dictyosomes.
2. During division, the dictyosomes appear as batonettes scattered throughout the cytoplasm around the spindle.
3. In late spermatids, the separate Golgi elements which have been scattered around the developing vesicle are aggregated into an acroblastic mass which lies in the lower part of the sperm.
4. The mitochondria occur throughout the maturation divisions and in the spermatids as irregular filaments. As the vesicle develops, the mitochondria form or are carried into its wall.
5. Two chromatoid bodies are present in early spermatocytes. They may be aggregated equally in the first maturation division or both may pass to one secondary spermatocyte. Where two chromatoid bodies exist in one spermatocyte, equal segregation to the spermatids seems to be the rule. These bodies cannot be interpreted as Golgi material.
6. Spermatid nuclei thin out in the center and presently show a definite central opening. The nucleus continues to appear as a ring throughout spermioteleosis and is ring-like in the sperm.

7. The proximal portions of the radial arms of the sperm are formed by extensions of the nuclear ring.
8. A compound centriole occurs in the spermatid. It migrates into the center of the nuclear ring and itself becomes a ring from which a fiber radiates into each arm.
9. A second centriole-like body makes its appearance in late spermatids and hollows out into a barrel-like structure which pushes its way into the base of the developing vesicle to form the inturned portion of the vesicular wall.
10. The crayfish sperm is essentially a radially arranged structure divisible into: a) an upper vesicle, b) a middle or nucleo-centrosomal portion, and c) a cytoplasmic cup underneath. These regions are not homologous as such with the head, mid-piece and tail of the flagellate sperm. Nevertheless the individual components of the crayfish sperm seem to be completely homologous with the corresponding components of the typical flagellate sperm.

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Chromosomenstudien an Orchidazeen. III. Über das Vorkommen von haploiden Pflanzen bei *Bletilla striata* Reichb. f. var. *gebina* Reichb. f.[1]

Von

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Eingegangen am 20. Januar 1940

Einleitung

Seit der Entdeckung der haploiden Pflanzen von *Datura stramonium* im Jahre 1922 durch Blakeslee, Belling, Farnham und Bergner, sind ziemlich viele haploide Pflanzen verschiedener Art gefunden worden und das Gebiet der Arten der gefundenen haploiden Pflanzen ist so groß geworden, daß es schon 14 Gattungen und 29 Arten umfasst. Demgemäß wurden die darauf bezüglichen Forschungen von verschiedenen Forschern schnell gefördert. Daß die Erforschung der haploiden Pflanzen eine wichtige Bedeutung bezüglich der Zellen- und Vererbungslehre hat, ist schon von vielen Forschern gesagt worden. Auch der Verfasser konnte haploide Pflanzen in Orchidazeen finden, in deren Familie bis heute keine haploiden Pflanzen gefunden worden sind und möchte hierüber berichten. In diesem Berichte aber schreibe ich hauptsächlich über die zytologischen Beobachtungen. Bezüglich der Einzelheiten der äußeren Gestalt, möchte ich später meine genauen Beobachtungen veröffentlichen.

Über die bisherigen Untersuchungen hat M. A. Ivanov (1938) eine eingehende Beschreibung mit Tabelle der bisher gefundenen haploiden Pflanzen veröffentlicht, und so möchte ich hier unterlassen darüber zu berichten.

Material und Methode

Die betreffenden haploiden Pflanzen von *Bletilla striata* Reichb. f. var. *gebina* Reich. f. wurden von Herrn K. Suzuki 1912 gezüchtet. Nämlich durch die Gattungskreuzung von *Bletilla striata* Reichb. f. var. *gebina*. Reichb. f. × *Eleorchis japonica* F. Maekawa hat er 1911 eine Kapsel der Mutterpflanze, *Bletilla striata* var. *gebina* geerntet. Im nächsten Jahre wurden viele Samen dieser Kapsel ausgesät, und

1) Contributions from the Divisions of Plant-Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 249.

einige F_1-Pflanzen sind jedes Jahr neu aufgeblüht, wenn sie auch hochprozentige Sterilität zeigten. Ein anderer Teil von F_1-Pflanzen aber, hat bis heute nie geblüht, trotzdem sie auf gleiche Art wie die anderen gezüchtet worden sind. Herr K. SUZUKI hat sie in zwei Gruppen eingeteilt, nämlich in eine blühende und eine nicht blühende Gruppe.

Dank der Freundlichkeit von Herrn K. SUZUKI, konnte der Verfasser diese zwei Gruppen von Pflanzen und ihre Mutterpflanzen für die vorliegenden Untersuchungen frei benutzen.

Auch Graf und Gräfin TOKUGAWA haben mir gütigst erlaubt, die Pflanzen von derselben Art wie die Mutterpflanzen für die vorliegenden Experimente frei und genügend in ihrem Garten zu fixieren.

Hier möchte ich Graf und Gräfin TOKUGAWA und Herrn K. SUZUKI meinen herzlichsten Dank für ihre Freundlichkeit aussprechen.

Die oben erwähnten zwei Gruppen der Pflanzen sind in Töpfe gesetzt worden, die Mutterpflanzen dagegen im Freien belassen. Zur Fixierung der Wurzeln und der Blumenknospen wurden zwei Lösungen gebraucht: Die NAWASCHINsche und die in dem Laboratorium der kaiserlichen Universität Hokkaidō modifizierte FLEMMINGsche Lösung. Die erstere Lösung aber hat besseren Erfolg gebracht. Nur bei der Fixierung der Blumenknospen wurde die Luftpumpe benutzt, um eine schnellere Eindringung der Fixierungsmittel zu erreichen. Alle Wurzelspitzen wurden 12–16 Mikron dick und die Blumenknospen 16–18 Mikron dick geschnitten und beide wurden nach NEWTONscher Jod-Gentianaviolett-Methode gefärbt.

In der vorliegenden Arbeit habe ich alle Zeichnungen mit dem Zeichen-apparat nach ABBE in 3.300-facher Vergrößerung gezeichnet und beim Druck zu der bei jeder Figur eingetragenen Größe eingeschränkt und alle Mikrofotografien mit Hilfe von LEITZ Makam aufgenommen.

Beobachtung

Somatische Chromosomenzahl 1937 hat der Verfasser die somatische Chromosomenzahl der oben erwähnten zwei Gruppen von Pflanzen an ihren Wurzeln untersucht und konnte bestätigen, daß alle Pflanzen der blühenden Gruppe eine Chromosomenzahl von $2n = 16$ haben und die nicht blühenden Pflanzen $2n = 36$ (Fig. 1 u. 2). Wie bereits veröffentlicht (MIDUNO 1939), ist die somatische Chromosomenzahl der Mutterpflanzen, *Bletilla striata* var. *gebina* 32 und die der Vaterpflanzen, *Eleorchis japonica* 40. Demgemäß ist die somatische Chromosomenzahl der Pflanzen der nicht blühenden

Gruppe die Summe der reduzierten Chromosomenzahl der Elternpflanzen (16 + 20 = 36).

In bezug auf die Blumenform und -farbe, die Blütezeit und die äußere Gestalt sind die Pflanzen mit 2n = 16 Chromosomen sehr ähnlich der von *Bletilla striata* var. *gebina*, nur daß jene, im Ganzen betrachtet, kleiner sind als diese . Die Knolle von *Eleorchis japonica* stirbt ab und entsteht jedes Jahr neu. Die von *Bletilla striata* var. *gebina* aber, stirbt nie ab und die neue Knolle entsteht auf der alten des vorigen Jahres, welche nicht abstirbt sondern jedes Jahr wieder sprosst. Bei den Pflanzen mit 2n = 16 Chromosomen stirbt die Knolle nicht ab wie bei den Mutterpflanzen, *Bletilla striata* var. *gebina*. Bei den Pflanzen mit 2n = 36 Chromosomen aber, ist dieses Verhältnis intermediär zwischen den beiden Elternpflanzen, nämlich die neue Knolle entsteht auf der des vorigen Jahres, welche aber nur zwei oder drei Jahre lang lebt und dann abstirbt.

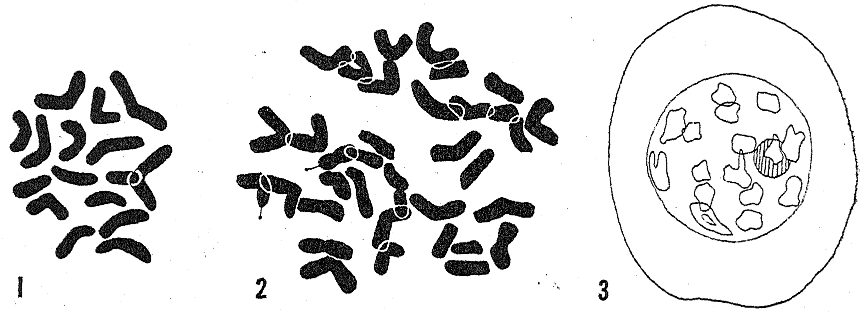


Fig. 1. Somatische Chromosomen der haploiden Pflanzen. 2n = 16 (3300×). Fig. 2. Somatische Chromosomen der Bastarde. 2n = 36 (3300×). Fig. 3. 16 Bivalenten in Diakinese. (*B. striata* var. *gebina*) (1650×).

Nach den oben erwähnten Chromosomenzahlen und den Erscheinungen darf man vermuten, daß die Pflanzen welche blühen, die durch den Reiz der Pollen von *Eleorchis japonica* parthenogenetisch entstandenen haploiden Pflanzen von *Bletilla striata* var. *gebina* sind und weiter daß die Pflanzen, welche nicht blühen, die wirklich befruchteten dibasischen Pflanzen, oder mit anderen Worten Bastardpflanzen sind.

HOLLINGSHEAD (1928) hat bei den haploiden Pflanzen von *Crepis* die Mixoploidie in den Wurzeln beobachtet, bei den betreffenden haploiden Pflanzen aber wurden keine Mixoploidie gefunden, trotzdem der Verfasser viele Präparate der Wurzeln untersucht hat.

Reduktionsteilung in *Bletilla striata* var. *gebina* Die Mikrosporogenese von *Bletilla striata* var. *gebina*, welche die Mutterpflanze

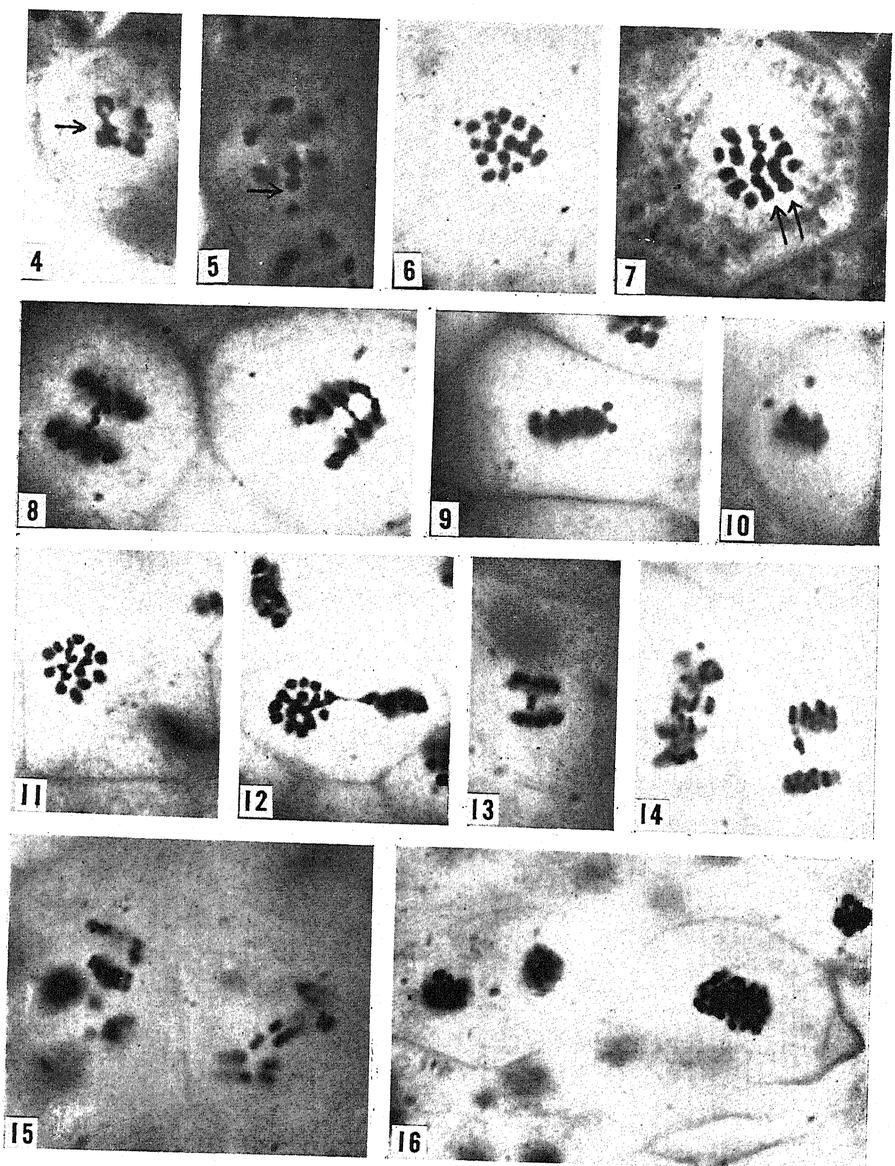
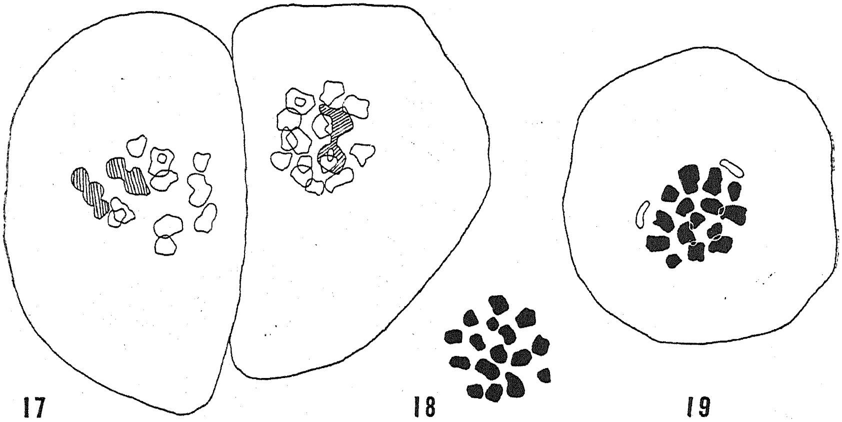


Fig. 4–16. *B. striata* var. *gebina* 1200×. 4, 5. Sekundärverbindung der Bivalenten (mit Pfeil gezeigt) in Pro-Metaphase (Fig. 4) und Diakinese (Fig. 5). 6. 16_{II} in IM. 7. IM. $2\cdot2_{II}+12\cdot1_{II}$. Die Sekundärverbindungen sind mit Pfeil gezeigt. 8. Chromatinbrücke in IA. 9. IM. Ein Bivalent teilt sich früher als andere. 10. IM. Zwei Univalenten gehen nach einem Pol über. 11. IIM. $1\cdot3_{I}+2\cdot2_{I}+9_{I}$. Zwischen sekundär verbundenen Chromosomen kann man die Verbindung durch eine kurze Chromatinbrück erkennen. Folglich können die sekundär verbundenen Chromosomen als Bivalenten oder als ein Trivalent angesehen werden (s. Fig. 20). 12. IIM. Die in IM entstandene Chromatinbrücke bleibt unverändert. 13. IIM. Ein retardierendes Chromosom. 14. u. 15. Die gestörten zweiten Teilungen. 16. IIM. Die normalen Kerne (links) und der Restitutionskern (rechts).

der haploiden Pflanzen ist, wurde beobachtet und gefunden, daß sie etwas unregelmäßig verläuft.

In Diakinese oder in Pro-Metaphase kann man zwei oder einige Bivalenten, welche miteinander in einer Reihe verbunden sind, sehen (Fig. 4 u. 17). Bei der Polansicht der Metaphase, sind 16 Bivalenten zu beobachten (Fig. 6 u. 18) und in den meisten Fällen wurde, wie in Diakinese oder in Pro-Metaphase die Verbindung der Bivalenten gefunden (Fig. 7). Diese Verbindung der bivalenten Chromosomen ist die Sekundärverbindung (Sekundärpaarung od. Sekundärkoppelung) und übt fast keinen Einfluß auf die erste Teilung aus, welche regelmäßig verläuft. Von Zeit zu Zeit kommen zwei Univalenten vor (Fig. 19). Ein bivalentes Chromosom aber teilt sich immer



17 18 19

Fig. 17. Die Sekundärverbindung in Pro-Metaphase. Links: $1 \cdot 2_{II}+14 \cdot 1_{II}$, rechts $1 \cdot 3_{II}+1 \cdot 2_{II}+10 \cdot 1_{II}$ (Die sekundär verbundenen Chromosomen sind mit schrägen Linien gezeichnet) (s. Fig. 4 u. 5). Fig. 18. IM. 16 Bivalenten. Fig. 19. IM. 15 Bivalenten und 2 Univalenten.

früher als andere und bei Seitenansicht können diese früher geteilten Chromosomen leicht als Univalenten verkannt werden (Fig. 9). Folglich ist es wahrscheinlich, daß die oben erwähnten zwei univalenten Chromosomen von diesem bivalenten Chromosom durch "vorzeitige Trennung" abstammen. In Fig. 10 aber, sieht man zwei Univalenten nach einem Pol übergehen. In diesem Fall müssen sie echte Univalenten sein.

Bei den Teilungen, kommen oft eine oder zwei Chromatinbrücken vor und deuten das Vorhandensein der interstitialen Chiasmata und der Inversion in den betreffenden Chromosomen an (Fig. 8).

Wie oben erwähnt wurde, verläuft die erste Teilung gewöhnlich regelmäßig, die zweite Teilung dagegen ziemlich unregelmäßig. Auch in IIM wurden 16 Chromosomen gezählt. Die sekundär ver-

bundenen Chromosomen aber, sind viel zahlreicher als in IM und nach ihrer Form zu beurteilen, scheint der Zustand der Verbindung fester zu sein (Fig. 11 u. 20). Retardierende Chromosomen wurden auch häufig gesehen (Fig. 13). Bei Orchideen entsteht nach der

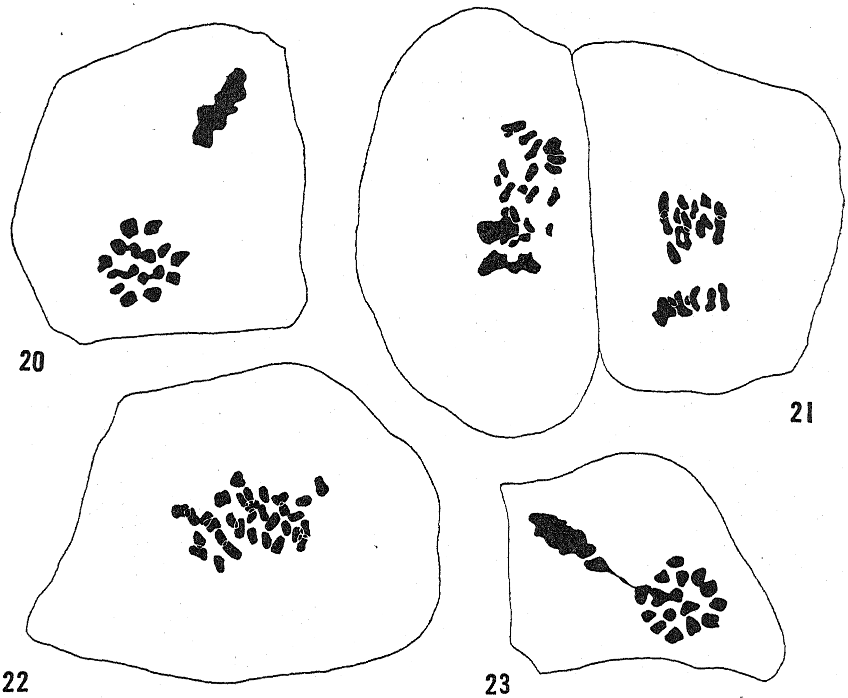


Fig. 20. Die Sekundärverbindung in IIM. $1 \cdot 3_{I} + 2 \cdot 2_{I} + 19 \cdot 1_{I}$ (s. Fig. 11). **Fig. 21.** Die gestörten zweiten Teilungen. **Fig. 22.** IIM. Eine Zelle mit unreduzierten Chromosomen (32). **Fig. 23.** Eine in IM entstandene Chromatinbrücke bleibt unverändert noch in IIM.

ersten Teilung keine Scheidewand zwischen beiden Tochterchromosomengruppen. Als Folge davon vereinigen sich oft die Spindeln und ziemlich viele 2n-chromosomige Pollen entstehen wie in Tabelle 1 gezeigt ist, oder die Teilung wird stark gestört und eine multipolarische Teilung ausgeführt (Fig. 14, 15 u. 21). Infolgedessen kommen die Pollen vor, welche von der normalen Chromosomenzahl stark abweichende Chromosomenzahl haben.

Tabelle 1

Chromosomenzahl in Pollenteilung	16 (n)	32 (2n)
Frequenz	118	18
Prozent	87%	13%

Selten kann man 2n-Chromosomen in IIM finden, vermutlich ist dies als Erfolg der Entstehung der Restitutionskerne in den ersten Teilungen, anzusehen (Fig. 22).

Wie in Fig. 12 und 23 gezeigt ist, bleibt oft die in der ersten Teilung entstandene Chromatinbrücke unverändert bis zur zweiten Teilung. Neulich haben G. L. STEBBINS Jr. und J. A. JENKINS die gleiche Erscheinung beobachtet und vermutet, daß diese Erscheinung wenigstens einen Teil der Ursache welche die Chromosomengruppen in II-Teilung vereinigen läßt, ausmacht. Wie sie erwähnt haben, entsteht die Chromatinbrücke hauptsächlich in verhältnismäßig kleinen PMZ, folglich kann die Vereinigung der Chromosomengruppen leicht vorkommen.

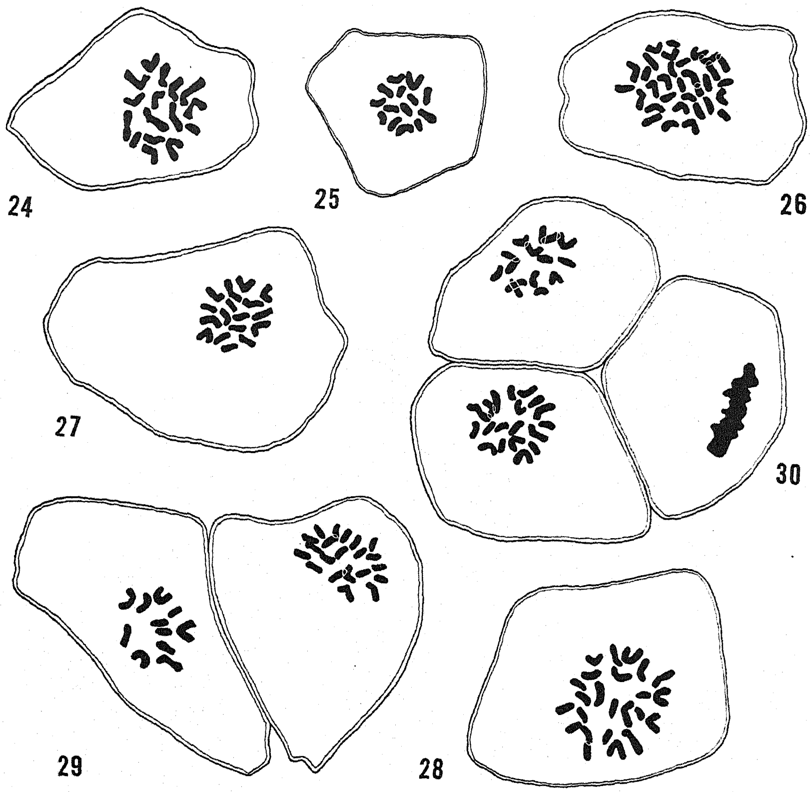


Fig. 24–30. Erste Kernsteilung in Pollenkörner (2200×). **24.** Normaler Pollen mit 16 Chromosomen. **25.** 14-chromosomiger Pollen. **26.** 32(2n)-chromosomiger Pollen. **27.** 17-chromosomiger Pollen. **28.** 24-chromosomiger Pollen. **29.** 21-chromosomiger (rechts) und 11-chromosomiger (links) Pollen. **30.** 19-chromosomiger (unten) und 15-chromosomiger (oben) Pollen.

Die Kernteilung in Pollenkörnern verläuft meistens normal. Pollenkörner mit verschiedenen Chromosomenzahlen, die durch die verschiedenen Abweichungen in I- und II-Teilungen entstanden sind, wurden beobachtet. Fig. 24–33, zeigen solche Pollenkörner.

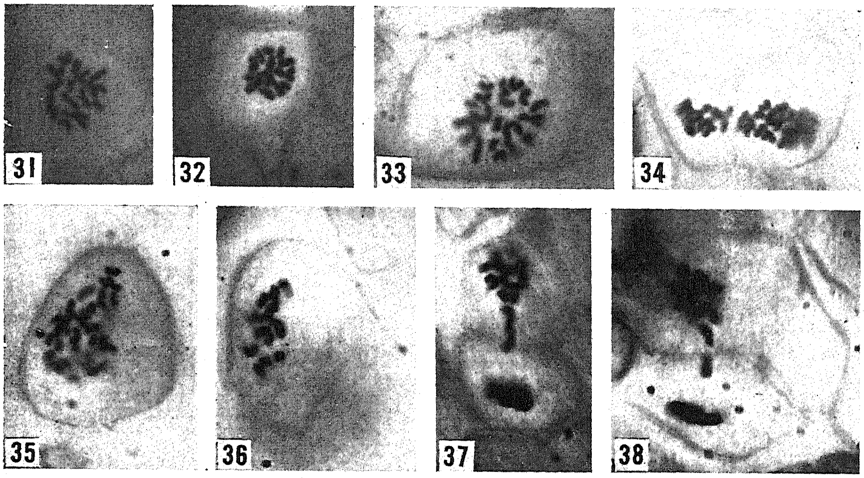


Fig. 31–38. Erste Kernteilung in Pollenkörner (1200×). **31.** 16-chromosomiger Pollen (normal). **32.** 14-chromosomiger Pollen (s. Fig. 25). **33.** 24-chromosomiger Pollen (s. Fig. 28). **34–36.** Pollen mit abweichender Chromosomenzahl. **Fig. 37–38.** Retardierende Chromosomen.

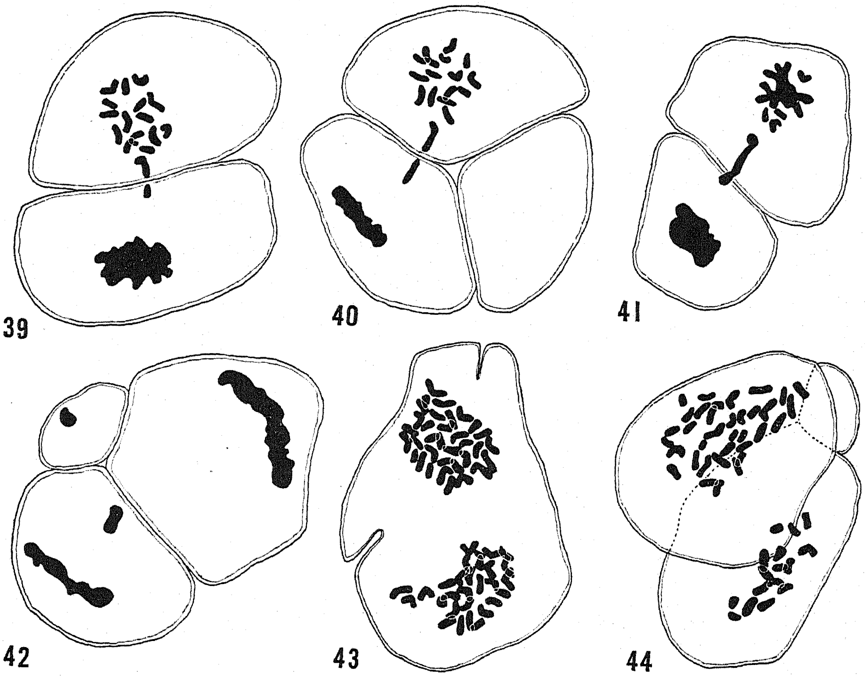


Fig. 39–41. Die in IIM entstandenen retardierenden Chromosomen. Ein Pollen hat 16 Chromosomen und eins von ihnen ist durch Zellwand in zwei ungleichmäßige Teile getrennt. **Fig. 42.** Pollen von abweichender Größe. **Fig. 43.** Pollen mit stark abweichenden Chromosomen in zwei Gruppen. Die obere Gruppe hat ungefähr 29 und die unte:e ungefähr 34 Chromosomen. **Fig. 44.** Pollen mit abnormer Chromosomenzahl.

Es ist sehr wahrscheinlich, daß die Pollenkörner mit den von den normalen Chromosomenzahlen nur wenig abweichenden Chromosomenzahlen durch die retardierenden Chromosomen in II-Teilung entstanden sind. Man kann solche retardierenden Chromosomen sehr gut beobachten wie sie in Fig. 37–41 zu sehen sind, weil die Tetraden bei den Pflanzen der Orchidazeen sich nicht trennen obgleich die Pollen vollkommen reif sind. Wenn solche retardierenden Chromosomen in der II-Teilung eliminieren, oder in einer Zelle der Tetraden enthalten sind, ist es sehr wahrscheinlich, daß Pollenkörner mit der von der normalen Chromosomenzahl wenig abweichenden Chromosomenzahl, vorkommen. Außerdem können vielleicht die Ungleichmäßigkeiten der Chromosomengarnitur durch eine ungleichmäßige Teilung der Chromosomen durch die Scheidewand entstehen, wie in Fig. 37, 39, 41 gezeigt ist. Weiter kann sich die ziemlich feste Verbindung der Chromosomen in II-Teilung, auf die Entstehung der Pollenkörner mit der beinahe normalen Chromosomenzahl beziehen. Wenn es der Fall ist, ist die Verbindung nicht mehr "Sekundärverbindung" sondern echte Verbindung vielleicht mit Chiasmata und die Chromosomen können als Bivalenten, Trivalenten, Tetravalenten oder Multivalenten angesehen werden. Diesbezüglich bedarf es weiterer Forschung.

Die retardierenden Chromosomen bilden oft die Mikronuklei und demgemäß die Polyaden (Fig. 42).

Die Pollenkörner mit stark abweichenden Chromosomenzahlen sind vielleicht dadurch entstanden, daß die beiden Chromosomengruppen bei II-Teilung sich vereinigen, und nachher die gestörten Verteilungen der Chromosomen stattfanden (Fig. 14, 21, 34, 35, 36, 43, 44).

Die oben erwähnten Pollenkörner mit verschiedenen Chromosomenzahlen haben vermutlich keine Befruchtungsfähigkeit. Die Beobachtung der reifen Pollen in Antheren aber, zeigt fast keine abortiven oder degenerierten Pollen, obwohl man Pollen von verschiedener Größe finden kann. Demgemäß scheinen auch die Pollen von verschiedenen Chromosomenzahlen den Verlauf der Bildung der vegetativen, und generativen Kerne vollenden zu können.

Die Beobachtung der Eizellen wurde in dieser Untersuchung nicht ausgeführt. Wenn die Abweichungen wie bei Mikrosporogenese auch bei Makrosporogenese entstehen, so ist es nicht unmöglich, daß Pflanzen mit abweichenden Chromosomen in den Nachkommen vorkommen. Im Freien aber, vermehrt sich *Bletilla striata* meistens vegetativ mit Knollen und es ist fast unmöglich, Pflanzen mit abweichenden Chromosomen durch Befruchtung hervor zu bringen.

Die Größe der Samen der betreffenden Pflanzen ist ziemlich ungleichmäßig. Dies scheint von den abnormen Erscheinungen in der Reifeteilung abhängig zu sein.

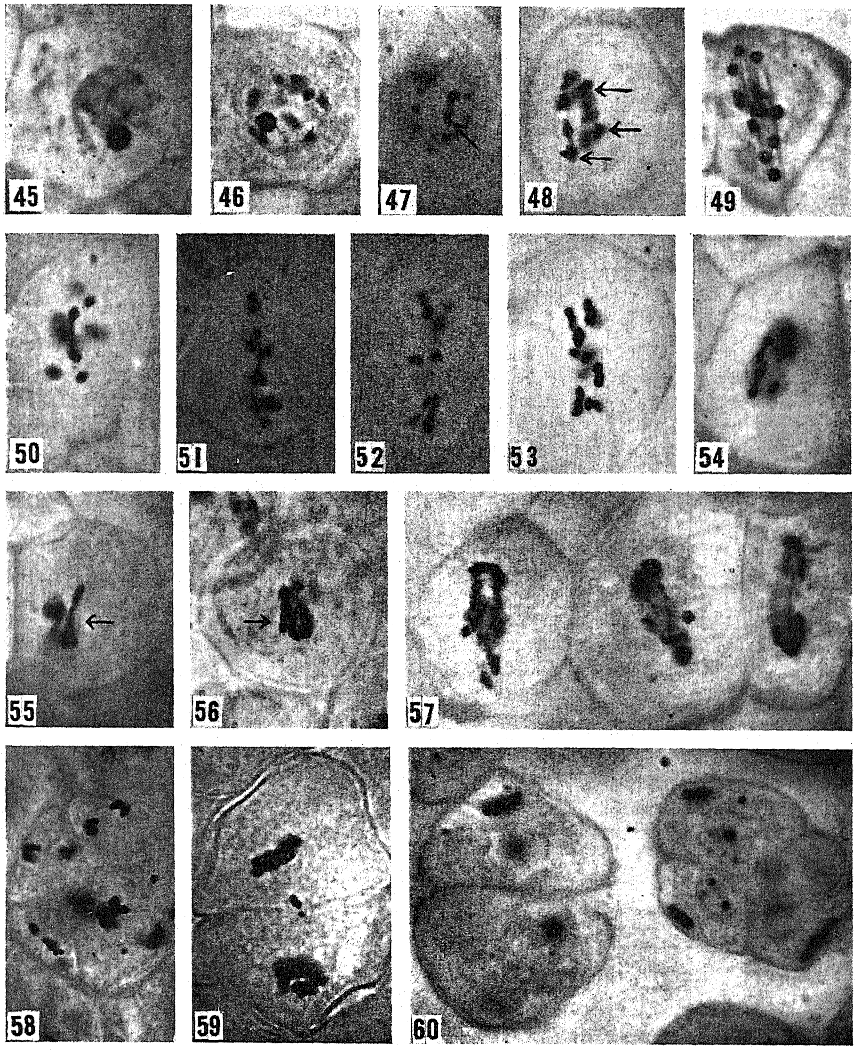


Fig. 45–60. Haploid-*Bletilla* (1200×). 45. Prophase. 46. Diakinese. 47. Diakinese. Man kann ein verbundenes Chromosom sehen (mit Pfeil gezeigt). (s. Fig. 61). 48. Pro-Metaphase. Die Verbindung der Chromosomen (mit Pfeil gezeigt) beginnt hauptsächlich in diesem Stadium. 49. Pseudo-Metaphase. Univalenten gehen nach beiden Polen nacheinander über. 50–54. Teilungen mit Bivalenten, Univalenten und sekundär verbundenen Chromosomen. 55. Ein Trivalent. 56. Verbindung von Vierergruppen in Kettenform. (Vielleicht Sekundärverbindung.) 57. Kernteilung in gestreckter Form. 58. Kernteilung in Pollenkörner. Sogar in Pollen mit sehr wenigen Chromosomen findet Teilung statt. 59. Ein retardierendes Chromosom. Chromosomen beider Zellen bilden Metaphase-Platten. 60. Tetraden und Diaden.

Der Verfasser hat in desem Jahr die Samen der betreffenden Pflanzen auf Agarboden ausgesät und konnte viele Keimlinge gewinnen. Später wird er den Bericht der karyologischen Beobachtungen dieser Keimlinge veröffentlichen können.

Reduktionsteilung der haploiden Pflanzen Die Chromosomen der Pflanzen der Gattung *Bletilla* sind sehr klein und deshalb ist eine ausführliche Beobachtung der Prophase (Fig. 45) unmöglich. Folglich wird beim vorliegenden Bericht die Beobachtung von der Diakinese aus beschrieben werden. Man kann in jeder Anthere der vorliegenden Haploidpflanzen die Pollenmutterzellen in verschiedenen Stadien finden. CHIPMAN und GOODSPEED (1922) haben bei *Nicotiana*-Haploidpflanzen dieselbe Erscheinung beobachtet.

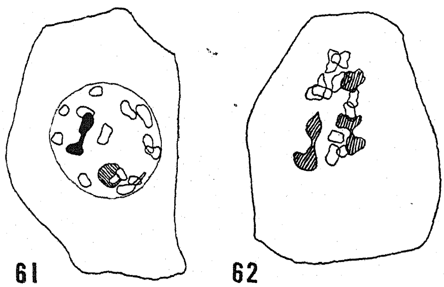

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Fig. 61. Verbindung der Chromosomen in Pro-Metaphase (schwarz gezeichnet) in Diakinese (s. Fig. 47). Fig. 62. Verbindung der Chromosomen in Pro-Metaphase (mit sehräger Linien gezeichnet) (s. Fig. 48).

In der frühen Diakinese sind 16 Univalenten zu sehen, welche mit gewöhnlichen feinen chromatischen Fäden miteinander verbunden sind. Im weiteren Verlauf des Stadiums verschwinden diese Fäden, doch alle Univalenten behalten an beiden Enden kurze Fädenreste, welche später verschwinden. In der Diakinese sind meistens keine Bivalenten zu beobachten (Fig. 46), gelegentlich aber bemerkt man die Existenz von 2–3 verbundenen Chromosomen (Fig. 47, 61). Manchmal sind zwei Univalenten mit den obenerwähnten Fäden sogar in der späteren Diakinese verbunden. Dieser Zustand aber scheint mir von den oben erwähnten verbundenen Chromosomen unterschiedlich zu sein. Die ungenügenden Materialien haben mich verhindert es quantitativ festzustellen, doch ist es unzweifelhaft, daß die Zahl der verbundenen Chromosomen in der Diakinese viel geringer als in der Metaphase ist. Diese Neigung ist auch in den bisher gefundenen haploiden Pflanzen oft beobachtet worden.

Die Verbindung der univalenten Chromosomen kommen in Pro-Metaphase vor (Fig. 48, 62) wie die Bivalenten in diploiden Pflanzen (Mutterpflanzen) in demselben Stadium sekundär verbunden werden. Diese Erscheinung scheint sehr wichtig zu sein in bezug auf das Problem der Sekundärverbindung.

Das Stadium der Metaphase ist bei der betreffenden Pflanze nicht klar. Wenn das Stadium von der Diakinese zu Metaphase

übergeht, kommen die Chromosomen sehr nah zusammen und drängen sich dicht aneinander. Die Zählung der Chromosomen in diesem Zustand ist unmöglich weil sie keine Kernplatte bilden. Dieses Stadium ist nämlich die sogenennte "Pseudo-Metaphase", welche für die meisten haploiden Pflanzen bezeichnend ist. Von diesem Zustand an beginnen die Chromosomen nach beiden Polen überzugehen aber nicht gleichzeitig, sonder nacheinander. Sehr selten kann man eine gebildete Kernplatte sehen. Aus obenerwähntem Grund mußten Zahl und Form der Chromosomen meistens in der frühen Anaphase beobachtet werden. In diesem Stadium liegen die Chromosomen in der Spindel zerstreut umher, und nach meiner Beobachtung kommen dann 16 univalente Chromosomen, oder einige verbundene Chromosomen von Zweier-, Dreier-, oder Vierergruppen zusammen mit einigen Univalenten vor. Das Verhältnis zwischen diesen beiden Fällen ist in jeder Anthere verschieden. In den meisten Kernen kommen die verbundenen und die univalenten Chromosomen gleichzeitig vor.

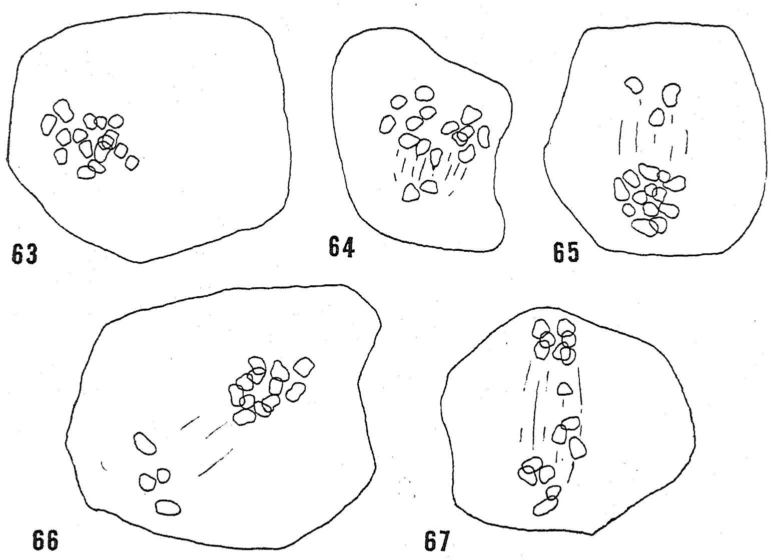


Fig. 63–67. Verschiedene Verteilungen der Univalenten in IM. von Haploid-*Bletilla* (2200×).

Dagegen nur wenig Kerne mit nur univalenten Chromosomen, und ihre Zahl ist bei jeder Anther unbestimmt. Vielleicht kann diese Unbeständigkeit der Zahl der verbundenen Chromosomen auf den Einfluß der Temperatur in der Zeit der Fixierung zurückgeführt werden.

Wenn nur Univalenten vorkommen ist ihre Verteilung nach beiden Polen zufällig, und man kann alle Verteilungen von 0–16 bis

8–8 Chromosomen finden (Fig. 49, 63–67). Die Chromosomen aber kommen an beiden Polen nicht gleichzeitig, sondern nacheinander an, und die Chromosomen, welche zuerst vor den andern am Pol angekommen sind, verdichten sich. Das hat die Zählung der Chromosomen sehr schwer gemacht. Die Form der Univalenten ist

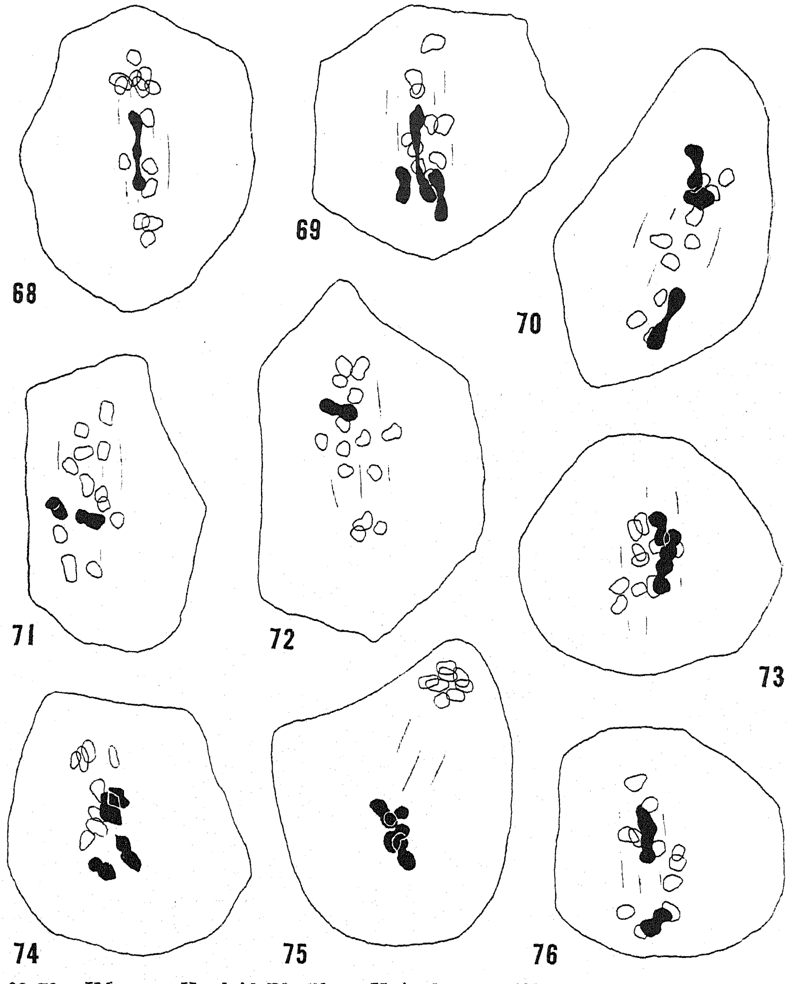


Fig. 68–76. IM von Haploid *Bletilla*. Univalenten, Bivalenten und Multivalenten kommen gleichzeitig vor. Hier ist kein Unterschied zwischen Bivalenten und sekundär verbundene Chromosomen, alle sind schwarz gezeichnet 68. $1_{II}+14_{I}$. 69. $3_{II}+10_{I}$, 70. $3_{II}+10_{I}$, 71. $2_{II}+12_{I}$, 72. $1_{II}+14_{I}$, 73. $1_{VI}+1_{II}+10_{I}$, 74. $4_{II}+8_{I}$, 75. Verteilung von 8_{I} und 4_{II}, 76. $1_{III}+1_{II}+11_{I}$. (Alle verbundene chromosomen werden als Bivalent, Trivalent oder Tetravalent angesehen).

meistens kugelförmig oder oval. Viele Univalenten sind in der Mitte etwas eingeschnürt und ziemlich ähnlich der Form der Bivalenten. Ihre Größe ist auch verhältnismäßig groß und haben

CHIPMAN und GOODSPEED (1922) dieselbe Erscheinung bei *Nicotiana tabacum* beobachtet. Die Längsspaltung der Univalenten bei IM ist fast nicht zu erkennen. Meistenteils sieht die Teilung so aus, als ob sie multipolarisch ausgeführt wäre. In Wirklichkeit aber, werden alle Chromosomen in beiden Tochterkernen eingeschlossen. Weiter kann man auch einige Univalenten finden, welche in, oder außer der Spindel bleiben, ohne von beiden Tochterkernen umschlossen zu werden. Diese Univalenten bilden Mikronuklei.

Falls verbundene Chromosomen zugleich mit Univalenten in IM vorkommen, teilen sich einige verbundene Chromosomen, einige andere aber, scheinen ungeteilt nach dem Pol überzugehen (Fig. 50–54, 68–76). Man muß die sich teilenden verbundenen Chromosomen als gewöhnliche Bivalenten ansehen, wenn sie auch am Pol, oder in der Nähe desselben liegen. Diese Bivalenten bilden verschiedene Formen und Trivalenten kommen selten vor (Fig. 55 u. 77). Es war aber sehr schwer zu unterscheiden, ob diese ungeteilten Chromosomen Bivalenten, oder sekundär verbundene Chromosomen waren, weil man die verschiedenen Stufen der Verlängerung zwischen sehr lang ausgedehente Bivalenten und sekundär verbundene Chromosomenpaare sehen konnte.

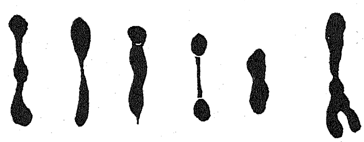


Fig. 77. Verschiedene Formen der Bivalenten und ein Trivalent. a–e: Bivalenten, F: Trivalent.

Als besondere Unregelmäßigkeit in der IM wurde der Zustand der Teilung, genau wie GAINES und AASE (1926) bei haploiden Pflanzen von *Triticum compactum humbolditte* gesehen und gezeichnet haben bestätigt (Fig. 9). Sie schreiben "Sometimes chromosomes seem to become entangled, the result being streched chromosomes extending between the nuclei; and this feature may become intensified until the chromosome groups are apparently torn apart into more or less irregular masses, often with connecting strands; or the entire chromosome group may cohere in an irregular mass in the center of the cell."

Nach obiger Feststellung ist das Verhalten der Chromosomen in der IM sehr kompliziert, denn gleichzeitig mit der gewöhnlichen Spaltung der Univalenten und der gewöhnlichen Teilung der Bivalenten, scheint in vielen Fällen eine Fragmentierung stattzufinden. Folglich wird die Form der einzelnen Chromosomen erstaunlich verwickelt und sehr viele Fragmente kommen vor. Wie GAINES und AASE geschrieben haben, koagieren die meisten Chromosomen in "unregelmäßigen Massen" und die Beobachtung des Verhaltens, oder der Form der einzelnen Chromosomen ist unmöglich in kurzen

Worten zu beschreiben. Das Verhältnis der an beiden Polen verteilten Chromosomenzahl ist auch vollkommen unklar. In der Anaphase kann man meistens die Chromatinbrücke zwischen der geteilten Chromatinmasse sehen. Diese Chromatinbrücke bleibt in der Telophase als Chromatinstück im Plasma und wird zuletzt durch die Zellmembran, welche zwischen den beiden Polen neu entsteht, in zwei Teile geschnitten. Meine Beobachtung stimmt mit der von GAINES und AASE überein auch in dem Punkt, daß "the entire chromosome group may cohere in an irregular mass in the center of the cell" und der Restitutionskern entsteht.

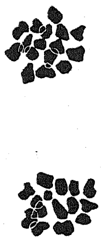
Ob die Ursache der Teilung dieser Art auf einem technischen Fehler beruht oder nicht, konnte ich nicht feststellen. GAINES und AASE haben auch nichts darüber geschrieben. Solche Teilung wurde auch nicht in diploiden Pflanzen beobachtet.

Fig. 78. Riesenzellen. Die größte Zelle hat vielleicht 4n Bivalenten und die Zelle der mittleren Größe 2n Bivalenten.

Weiter konnte der Verfasser einige Riesenpollenmutterzellen mit b, 2b und wahrscheinlich 4b Bivalenten beobachten (Fig. 78, 79, 80). Vielleicht kann man die Ursache ihrer Entstehung darauf zurückführen, daß die Restitutionskerne bei pro-meiotischen Teilungen in einigen Zellen entstanden sind und zwar bei den mit b Bivalenten einmal, und bei den mit 2b Bivalenten zweimal u. s. w..

Für eine eingehende Untersuchung der zweiten Teilung waren zu wenig Materialien vorhanden. Die Diaden, Triaden und Polyaden mit Mikronuklei aber zeigen eine Unregelmäßigkeit der zweiten Teilung.

Die Teilung in Pollen: Die Teilung in Pollen wurde sehr klar beobachtet. Es ist sehr merkwürdig, daß in allen Pollen, wenn sie auch sehr wenige Chromosomen haben, die Teilung ausgeführt werden konnte (Fig. 58, 81, 82). In sehr kleinen Pollen kann man nämlich oft 2 oder 3 Chromosomen im Stadium der Metaphase oder der Anaphase sehen. Bei vielen Pflanzenarten der Orchidazeen bleibt eigentlich jede Zelle der Tetraden oder der Polyaden, die von einer Pollenmutterzelle entstanden ist ungetrennt, und diese Pollen bilden ein Klümpchen, sogar nach der vollständigen Gestaltung aller Pollen. Weiter wird die Teilung in solchen Pollen gleichzeitig durchgeführt.



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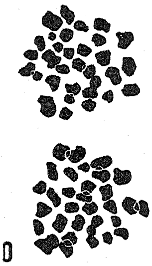

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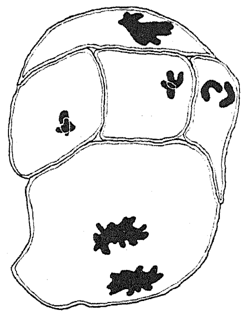

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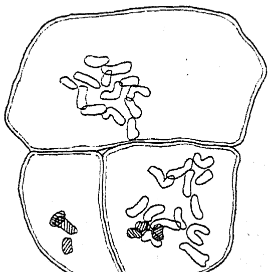

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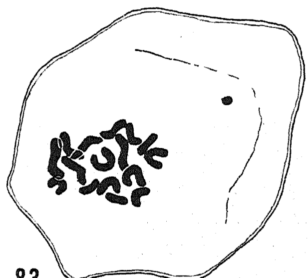

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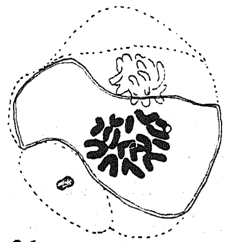

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Fig. 79. 16 Bivalenten in IM. **Fig. 80.** 32 Bivalenten in IM. **Fig. 81.** Erste Kernteilung in Pollenkörner. Auch Mikronuklei mit nur 2 Chromosomen führt Kernteilung aus. **Fig. 82.** Ein durch die Teilung von 13:3 entstandenes Tetrad. Zwei Zellen haben 13 und die beiden anderen 3 Chromosomen. **Fig. 83.** Pollen mit 16 Chromosomen und einem kleinen Fragment. **Fig. 84.** Pollen mit 16 Chromosomen und einer kleinen Zelle mit einem Chromosom, welches degeneriert zu sein scheint.

Folglich muß die Gesammtsumme der Chromosomenzahl in jeder Zelle einer Polyade selbstverständlich 32 sein. Doch durch das Vorkommen ziemlich vieler großer Fragmente ist die Zahl der Chromosomen oft mehr als 32.

Weiter kamen Pollen mit 16 Chromosomen selten vor (Fig. 83). Sie sind vielleicht durch die 0:16 Verteilung der Chromosomen bei der IM entstanden, und nur solche Pollen haben eine Befruchtungsfähigkeit. Desgleichen konnte der Verfasser Triaden bestehend aus zwei Pollen

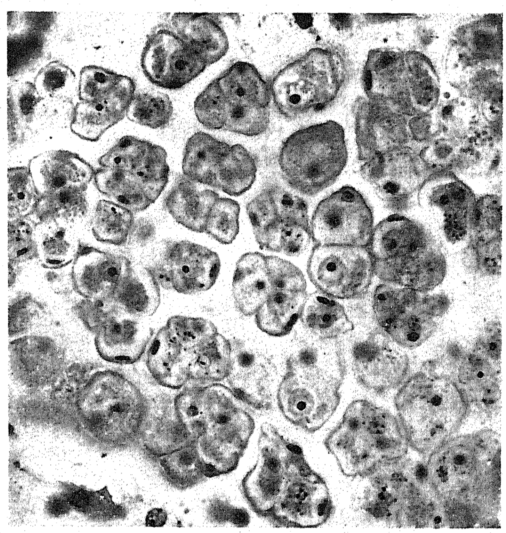

Fig 85. Alle Pollen von verschiedener Größe haben generative und vegetative Kerne (480×).

mit 16 Chromosomen und einer Pollen mit einem Fragment feststellen (Fig. 84). Nach ihrer Form zu urteilen, sind diese Fragmente vielleicht bei der IM entstanden und unverändert geblieben.

Was oben beschrieben wurde, ist die Beobachtung über die Metaphase der Kernteilung in Pollen. Es ist aber sehr merkwürdig, daß nach der Teilung, generative und vegetative Kerne in fast allen Pollenkörner gebildet werden (Fig. 85). Natürlich bei Diaden werden deutlich generative und vegetative Kerne gesehen. Sie sind vielleicht die durch 0:16 Verteilung entstandenen Pollen mit einem vollkommenen Satz.

Schlußbemerkung

Die oben erwähnten verschiedenen Erscheinungen sind alle den bisher gefundenen haploiden Pflanzen eigentümlich. Nämlich bei fast allen haploiden Pflanzen ist der Unterschied zwischen Diakinese und Metaphase undeutlich, die Univalenten liegen in der Spindel zerstreut umher und weiter wird die Verteilung der Univalenten zufällig ausgeführt. Folglich ist es nicht zu bezweifeln, daß die betreffenden Pflanzen haploide Pflanzen sind.

In der IM aber kommen ziemlich viele verbundenen Chromosomen von Zweier-, Dreier- oder Vierergruppen vor (Fig. 56, 73, 76). Sie sind alle kettenförmig. CATCHESIDE (1932) hat bei *Oenothera*-Haploidpflanzen eine ziemlich hochprozentige (20%) Bivalentenzahl und die ihr entsprechenden Multivalenten bei den diploiden Pflanzen beobachtet. Nach ihm beweist die Verbindung der Chromosomen in diesen haploiden Pflanzen die Existenz von verdoppelten Segmenten in einer Chromosomengarnitur. EMERSON (1929) hat auch in der haploiden *Oenothera-Pflanze* zwei Chromosomen sich oft paaren gesehen. Er deutete an, daß diese zwei Chromosomen vielleicht aus den vier Chromosomen stammen, welche in den Muterpflanzen einen Ring bilden. Bei den haploiden Pflanzen von *Nicotiana tabacum*, haben CHIPMAN und GOODSPEED (1922) wenig Bivalenten und Trivalenten gefunden und haben die Ursache ihres Vorkommens auf den mechanischen Effekt zurückgeführt. Auch LAMMERTS (1934) hat bei den haploiden Pflanzen von *Nicotiana tabacum* die stabförmigen Bivalenten mit einem terminalen, subterminalen oder interstitialen Chiasmata beobachtet. Er hat die Entstehung dieser stabförmigen Bivalenten auf "non-homologous association" zurückgeführt. MORINAGA und FUKUSHIMA (1933) haben bei den haploiden Pflanzen von *Brassicca napella* (2n = 19) 0–7 Bivalenten beobachtet. Diese haploide Pflanze von *Brassicca napella* ist eine digenomische Art, welche aus zwei Genomen, nämlich aus Genom "a" mit 10 Chromosomen und Genom "c" mit 9 Chromosomen besteht. Beide Verfasser

haben die Ursache des Vorkommens der bivalenten Chromosomen in der schwachen Affinität dieser beiden Genomen gesucht. YAMASAKI (1936) hat bei *Triticum vulgare*-Haploidpflanzen ($2n = 21$) einige Bivalenten und ein trivalentes Chromosom gesehen. Nach ihm geben Bivalenten, besonders ein trivalentes Chromosom einen Hinweis, um die Beziehung unter A-, B-, und D-Genom, welche die Chromosomengarnitur dieser haploiden Pflanzen bildet, deutlich zu machen.

Bei den betreffenden haploiden Pflanzen, scheint das Vorkommen der Bivalenten, Trivalenten und der kettenförmig in Zweier- Dreier- und Vierergruppen verbundenen Chromosomen, welche eine Sekundärverbindung vermuten lassen, im Verhältnis der Sekundärverbindung der Mutterpflanzen zu stehen.

Wie schon oben erwähnt wurde, kann man die Sekundärverbindung von zwei, oder mehreren Bivalenten bei der Reduktionsteilung der Mutterpflanzen, *Bletilla striata* var. *gebina* sehen.

Diese Sekundärverbindung der bivalenten Chromosomen in IM. scheint nicht so fest zu sein. Auch die Verteilung der Chromosomen in IM wird nicht gestört wie es in Sekundärverbindung immer der Fall ist. Gerade nach der Teilung der Chromosomen, kann man in beiden Chromosomengruppen jedes entsprechende Chromosom wie in Fig. 86 gezeigt ist, erkennen. In solchem Zustand, kann man fast keine Verbindung der Chromosomen finden, obgleich viele Verbindungen in IM in demselben Präparat beobachtet werden.

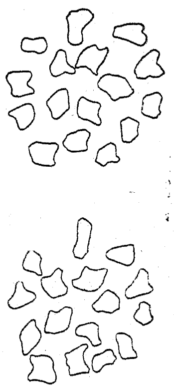

Fig. 86. Beide Kernplatten gerade nach IM. (*B. striata.* var. *gebina*). Die Chromosomen zeigen keine Sekundärverbindung (1500×).

Die Zeit in welcher die Verbindungen stattgefunden haben scheint die Pro-Metaphase zu sein. Nämlich in Diakinese findet man nur wenige Verbindungen im Vergleich mit Metaphase. In der Zeit, wenn die Chromosomen beginnen in die Kernplatte überzugehen, werden ziemlich viele verbundene Bivalenten gesehen (Fig. 4, 5, u. 17).

In IIM der Mutterpflanzen scheint der Zustand der Sekundärverbindung fester als in IM zu sein. Nämlich die verbundenen Chromosomen sind am Teil der Verbindung etwas ausgedehnt und scheinen eine kurze Chromosomenbrücke zu haben. Folglich sind ihre Formen mannigfaltiger als die der Chromosomen welche nicht verbunden sind. In Fig. 11 und 20 kann man die oben erwähnte kurze Chromosomenbrücke und das besonders lang ausgedehnte Chromosom sehen, welches man als

Bivalenten ansehen muß.

Bei den betreffenden haploiden Pflanzen, gehen oft einige verbundene Chromosomen nach einem Pol über, ohne sich zu trennen. Sogar die Verteilung 4_{II}: 8 wurde gefunden (Fig. 75). Außerdem kann man Bivalenten finden welche am Pol liegen und auf dem Wege der Teilung sind. Die Zeit der Teilung der Bivalenten in einer Zelle ist ungleich. Demgemäß sind die Formen der Bivalenten nicht bestimmt; einige sind in voller Länge andere aber, nur wenig verlängert (Fig. 50, 51, 52 u. 69) und diese Zustände machen eine Unterscheidung zwischen den sekundär verbundenen Chromosomen und Bivalenten schwer.

Die Zahlen der Bivalenten und der sekundär verbundenen Chromosomen sind von Zelle zu Zelle unbestimmt. Wenn viele sekundär verbundene Chromosomen vorkommen, gibt es allgemein nur wenige Bivalenten und umgekehrt.

Von den oben erwähnten Erscheinungen kann man vermuten, daß die Chromosomen je nach Umständen in Prophase und Pro-Metaphase, bivalente oder sekundär verbundene Chromosomen bilden können. Weiter entsprechen vermutlich die bivalenten und die sekundär verbundenen Chromosomen in IM der haploiden Pflanzen, den sekundär verbundenen Chromosomen in IM und IIM der Mutterpflanzen. Um diese Vermutungen sicher zu stellen bemüht sich der Verfasser jetzt eine genauere und quantitativere Untersuchung zu machen.

Die Ursache der Sekundärverbindung wurde schon von vielen Forschern untersucht, die Feststellungen aber über dieselben, sind noch nicht übereinstimmend. Die Forschung der Beziehungen zwischen haploiden und diploiden Pflanzen derselben Arten werden den Hinweis über die Ursache der Sekundärverbindung geben können. Weiter ist es sehr interessant, daß bei der Gattungskreuzung zwischen *Bletilla striata* var. *gebina* und *Eleorchis japonica* die haploiden Pflanzen der Mutterpflanzen entstanden und zugleich mit ihnen die Bastarde. Ein ähnliches Beispiel wurde bisher nicht gefunden. Bei den bisher gefundenen digenomischen oder trigenomischen Pflanzen sind schon die Geschlechtszellen der Elternpflanzen digenomisch oder trigenomisch und durch deren parthenogenetische Entwicklung entstanden. Demgemäß ist es denkbar, daß sie die Fähigkeit zu blühen haben.

Die Befruchtungsfähigkeit zwischen beiden Elternpflanzen deutet die Existenz der Affinität zwischen ihnen an, nämlich zwischen *Bletilla striata* var. *gebina* und *Eleorchis japonica* und zugleich daß das Zusammensein der einzelnen Sätze der Elternpflanzen, wenn sie auch vollkommen sind, dennoch ungeeignet für die Reifung, oder

für das Blühen sind. Mit anderen Worten, die Elternpflanzen sind in solchem Grade verwandt, daß sie wohl befruchtungsfähig, aber ihre F_I-Pflanzen nicht blühfähig sind; Weiter die haploiden Pflanzen haben genügend Vitalität um blühen zu können, wenn sie auch steril sind, und zeigen den Wert eines einzigen Satzes.

Zusammenfassung

1) Durch die Kreuzung *Bletilla striata* var. *gebina* (2n = 32) × *Eleorchis japonica* (2n = 40), sind die haploiden Pflanzen (2n = 16) von *Bletilla striata* var. *gebina* vielleicht durch die parthenogenetische Entwicklung und gleichzeitig auch die Bastarde (2n = 36) der Elternpflanzen entstanden.

2) Die haploiden Pflanzen haben nach 20 Jahren seit dem Säen zu blühen begonnen, sind jedoch fast vollkommen steril, die Bastarde dagegen haben nie geblüht.

3) Die Reduktionsteilung der Mutterpflanzen (*Bletilla striata* var. *gebina*) verläuft etwas unregelmäßig. Demgemäß kommen Pollen mit verschiedenen Chromosomenzahlen vor. Selten wurden einige Univalenten gefunden.

4) In IM und IIM der Mutterpflanzen, wurde die Verbindung einiger Bivalenten in Zweier-, Dreier- und Vierergruppen beobachtet. Diese Erscheinung mag als "Sekundärverbindung" angesehen werden.

5) Die Verbindung in IIM ist sehr fest und die verbundenen Chromosomen erschienen wie Bivalenten, Trivalenten oder Tetravalenten in Kettenform.

6) Die Reduktionsteilung der haploiden Pflanzen zeigt dieselbe Eigentümlichkeit, welche bei fast allen bisher gefundenen haploiden Pflanzen ausgedrückt ist.

7) In IM hat der Verfasser zwei Fälle gefunden: nämlich im ersten Fall, in welchem nur Univalente d. h. 16 Univalenten vorkommen, und im anderen Fall in welchem gleichzeitig Univalenten und Bivalenten vorkommen. Die ersten Fälle sind viel seltner als die zweiten.

8) Unter den Bivalenten gibt es zwei Arten. Eine zeigt echte Bivalenten und teilt sich normal in IM. Die andere zeigt sekundär verbundene Chromosomen, welche zu den Polen gehen ohne sich zu teilen. Bei letzterer Art findet man die Verbindung von Zweier-, Dreier- oder Vierergruppen der Chromosomen, alle in Kettenform.

9) Die Unregelmäßigkeit der Reduktionsteilung erzeugt Pollen mit verschiedenen Chromosomenzahlen. Die erste Teilung der

Pollenkörner findet in allen Pollen statt, wenn sie auch sehr wenige Chromosomen (zwei) haben.

10) Der Verfasser hat angedeutet, daß die sekundär verbundenen Bivalenten der Mutterpflanzen zu den Bivalenten und sekundär verbundene Chromosomen der haploiden Pflanzen gewisse quantitative Beziehungen haben. Er hofft es noch weiter zu erforschen.

Zum Schluß sei mir gestattet, meinem hochverehrten Lehrer Herrn Prof. Dr. Y. SINOTÔ für seine freundliche Hinweise, Besserungs- oder Ergänzungsvorschläge an dieser Stelle meinen verbindlichsten Dank auszusprechen.

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Studies in the Capparidaceae
VIII. The cytology of *Capparis zeylanica* Linn. and related genera

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I. Introduction

This is the genus after which the family is named. It is representative of the subfamily Capparidioideae. Cytological work on the family is very meagre indeed and that pertaining to this subfamily is confined to chromosome counts in five species of this genus, one of which was by the senior author on *Capparis sepiaria* (Raghavan 1938). Recently we described the cytology of *Crataeva religiosa,* another member of the Capparidioideae (Raghavan & Venkatasubban 1939) and recorded the presence of secondary association. Besides these there is no cytological work available on the Capparidioideae.

In the present paper, haploid chromosome counts have been made of three members of the subfamily for the first time, *Capparis zeylanica, Cadaba indica* and *Maerua arenaria.* In *Capparis zeylanica,* meiosis has been described in some detail especially as regards secondary association and in *Cadaba indica* material of which was not available in sufficient quantities for more detailed study, only the meiotic chromosome number has been reported. So also in the other genus.

In all these, it must be said that securing good cytological preparations was a matter of extreme difficulty. It is difficult enough

in the Capparidaceae as a whole, but it would appear to be especially so in this subfamily.

Material for this study was obtained from plants grown in the University Botanical Garden. *Capparis zeylanica* Linn., which is synonymous with *Capparis horrida* Linn., is a woody straggling shrub with prominent recurved thorns which are homologous with stipules. The presence of accessory buds and consequent occurrence of extra axillary flower buds is a feature worthy of note.

Anthers of the right stage of development previously determined by acetocarmine examination were fixed in Navashin's fluid after prefixation in Carnoy. Fixation of entire flower buds even after the removal of the calyx would not yield any good results. The deletion of prefixation was attended by certain failure to get any preparation worth the name. Embedding was done in paraffin in the usual way after the chloroform technique, and Newton's Iodine gentian violet was exclusively used for staining. The average thickness of the sections was about ten microns.

II. Observations

a. *Capparis zeylanica* Linn.

Stages earlier than First Metaphase were not studied in any detail on account of the extremely small size of the chromosomes. But even at diakinesis it could be clearly seen that the bivalents approximated closely to one another into separate groups. The most prominent feature of the First Metaphase was the occurrence of secondary association. As a matter of fact this phenomenon was so strongly in evidence that hardly a plate existed but exhibited secondary pairing. This is also corroborated by the accompanying table giving an analysis of the various associations, which would reveal that the range of variation of the number of associations is so small and between such high numbers as 7 and 13. The number of bivalents at MI is 20. Figures 1–12 are those of various MI plates showing different degrees of secondary association. Table A gives a summary of the various associations met with.

The maximum association is 3(4), 1(3), 2(2), and 1(1); and this was met with only once.

Secondary pairing is solely dependent on the diakinesis position of the bivalents relative to one another, and therefore all bivalents that lie adjacent at diakinesis and which are capable of secondary pairing are so paired at MI. As already indicated, at diakinesis itself, the bivalents could be seen to be closely approximated and it is these that constitute the secondary groupings at MI.

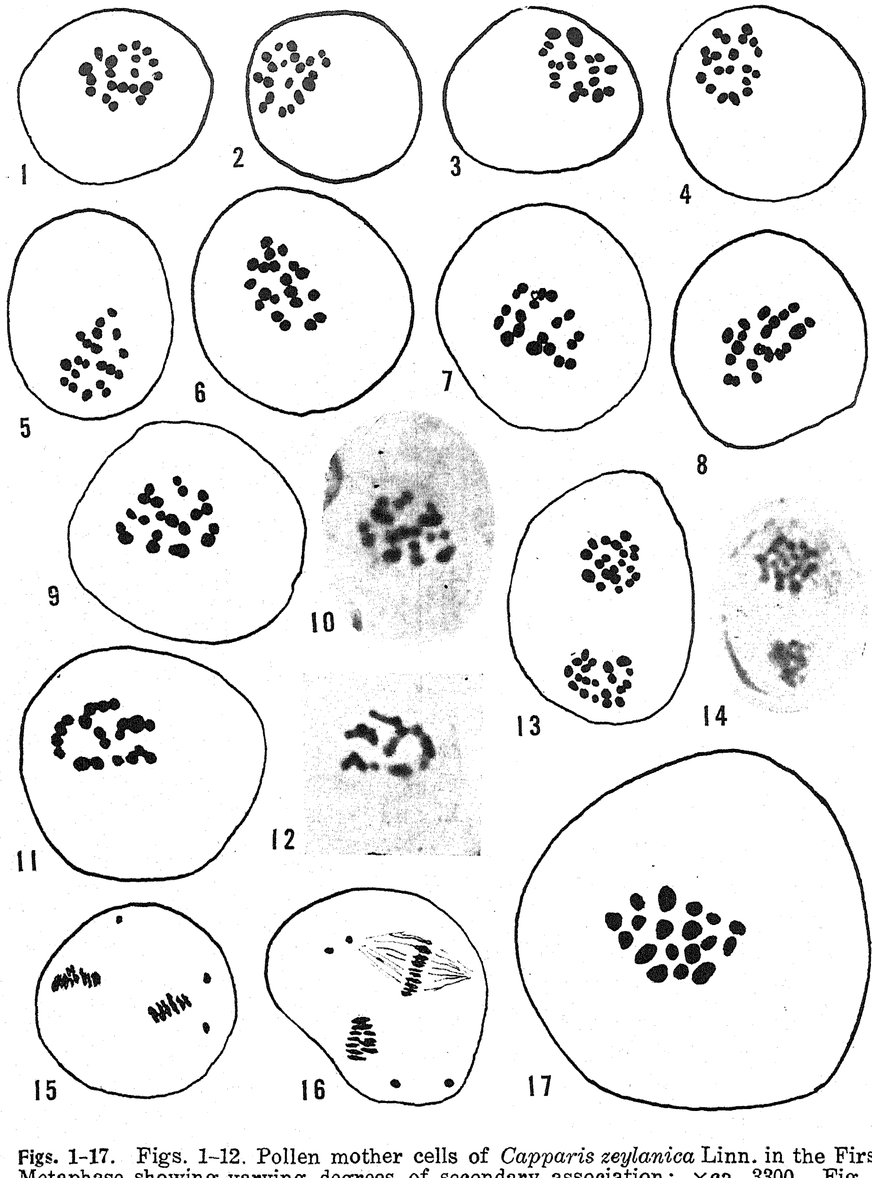
An interesting feature of this genus not recorded either in *Crataeva* or *Gynandropsis*, is that associations of four are very common. Another feature is the association between bivalents of dissimilar sizes (Figs. 1, 2, 7, 8, 11). This would indicate an affinity between non-homologous chromosomes which have some segments in common.

Table A

No. of associations	No. of bivalents in assn. 1	2	3	4	No. of cases	Total
7	8	3	2	—	1)	2
	7	5	1	—	1)	
8	4	8	—	—	1)	
	6	4	2	—	2)	8
	5	6	1	—	4)	
	7	2	3	—	1)	
9	3	7	1	—	2)	
	4	5	2	—	2)	6
	5	3	3	—	1)	
	2	9	—	—	1)	
10	2	6	2	—	4)	
	3	5	1	1	2)	8
	4	3	2	1	1)	
	3	4	3	—	1)	
11	1	5	3	—	4)	
	2	5	—	2	1)	6
	2	4	2	1	1)	
13	1	2	1	3	1	1
					Total	= 31

First division is not followed by wall formation. There is an interkinesis when the nuclear membrane is quite in evidence and the chromosomes are distributed more or less peripherally. Interkinesis is of very short duration and is immediately followed by second metaphase when the chromosomes lose their peripheral distribution and arrange themselves as a flat plate. The nuclear membrane has disappeared by this time (Figs. 13 and 14). Secondary association between chromosomes still persists. Figs. 13 and 14 represent the chromosome groupings. The same considerations that apply for the First Metaphase approximation of bivalents would appear to govern the groupings of MII chromosomes also. Enough MII plates were not available to make a comparative study of the groupings at this stage and those of MI. But Catcheside (1937) has made such a statistical study and found that MII plates show various degrees of secondary association covering the same range of types as at MI and in similar proportion.

During Second Metaphase there is not infrequently seen extrusion of variable number of chromosomes into the cytoplasm. Fig. 15 shows three having been extruded while in Fig. 16 four such bodies are seen. Presumably these will not be included in the tetrads to be formed and therefore they will be deficient. Naturally some proportion of the pollen grains would degenerate. Though division is on the whole normal, tetrads were seen to degenerate in a number of cases. Obviously these degenerations are a result of the deletions referred to.



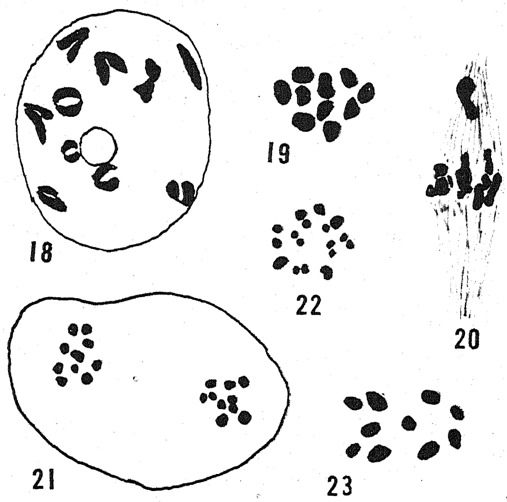
Figs. 1-17. Figs. 1-12. Pollen mother cells of *Capparis zeylanica* Linn. in the First Metaphase showing varying degrees of secondary association; ×ca. 3300. Fig. 1 shows 2(3) 3(2) 8(1). Figs. 2 and 3 show 8(2), 4(1); 1(3) 6(2) and 5(1) configuration. Figs. 4 and 5 show 1(3) 7(2) 3(1); 2(3) 5(2) and 4(1). Figs. 6 and 7 show 3(3) 4(2) 3(1); 2(3) 6(2) and 2(1). Figs. 8 and 9 show an association respectively of 2(4) 5(2) 2(1); 3(3) 5(2) 1(1). Fig. 10. Photomicrograph; Same as Fig. 9 showing the various groupings. Fig. 11 shows the maximum association of 3(4) 1(3) 2(2) and 1(1). Fig. 12. Photomicrograph of the same. Fig. 13. Second Metaphase showing the persistence of secondary pairing. ×ca. 3300. Fig. 14. Photomicrogragh of another Second Metaphase plate. Note the groupings of chromosomes. ×ca. 3300. Figs. 15 and 16. Second Metaphase showing the extruded chromosomes. In Fig. 15 there are 3 while in 16 there are 4. ×ca. 3300. Fig. 17. Pollen mother cell of *Cadaba indica* Lamk. in the First Metaphase showing 18 bivalents. Note the different sizes of the bivalents. ×ca. 5000.

In a plant growing in a locality close by, degenerations are extensive. There is practically no seed formation and when we investigated the plant it was found that pollen formation was very scarce. Degenerations set in at all stages of pollen development. Even as early as the differentiation of the microsporogenous cells whole anther loculi were seen to degenerate *en masse*. It would therefore appear that in this species there is a tendency towards extensive degeneration.

b. *Cadaba indica* Lamk., *Maerua arenaria* Hook. f. and Thomp., etc.

In addition to *Capparis zeylanica* dealt with in this paper, chromosome numbers of two important genera have been determined for the first time. Fig. 17 shows the p.m.c., of *Cadaba indica* in M.I. The haploid number is 18. An interesting feature in this is the varying size of the bivalents indicating that in the somatic complements there should be chromosomes of different sizes.

In *Maerua arenaria*, the other genus, the haploid number is 10. Fig. 18 shows a p.m.c. in diakinesis. The 10 bivalents are distributed peripherally. Most of these are of the rod type. Fig. 19 is an M I plate. Disjunction is normal. Occasionally cases of non-disjunction occur. In Fig. 20 a bivalent is seen to reach the pole earlier without having undergone separation. At MII the 10/10 distribution is almost the rule (Fig. 21) except in rare cases where slight variations are met with obviously due to the non-disjunction mentioned above.



Figs. 18-23. Figs. 18–21. *Maerua arenaria*. Fig. 18. p.m.c of *Maerua arenaria*-diakinesis; the 10 bivalents are distributed peripherally. Fig. 19. p.m.c M I (n=10). Fig. 20. p.m.c A I; a case of non-disjunction of a bivalent. Fig. 21. p.m.c M II; 10/10 distribution. Fig. 22. p.m.c of *Cleome Chelidonii*—M I; (n=17). Fig. 23.p.m.c of *Cleome viscosa*—M I; (n=10).

In a previous paper (Raghavan 1937) the chromosome number of *Cleome Chelidonii*, based upon aceto-carmine smears was tentatively given as ten. It was not possible then to confirm it by further fixation. Now extensive fixation of the anthers of this species was

rendered possible on account of a plentiful availability of material and it is seen that the haploid number is 17 (Fig. 22). It is quite likely that in the previously examined aceto-carmine preparation, the full number could not be counted presumably because of secondary association of bivalents, a phenomenon which has been found to be very widely prevalent in almost all the members of the Capparidaceae examined so far. It is of interest also to record here that in the closely related *Gynandropsis pentaphylla,* where also the haploid chromosome number is 17, the frequency of occurrence of secondarily paired vibalents in groups of 10, was found to be almost at the region of the mode. It is not therefore unlikely that the few plates examined in aceto-carmine showed the modal groupings and the approximation is so close that groups could easily be mistaken for individual bivalents.

The haploid number of *Cleome viscosa* Linn. was confirmed to be 10. (Fig. 23).

It is interesting that though *Gynandropsis pentaphylla* and *Cleome viscosa* are so strikingly similar morphologically, their chromosome number is so different; whereas *Cleome Chelidonii* whose resemblance to *Gynandropsis* is very much less, shows the same chromosome number. Similarly, *Maerua* a member of the Capparidioideae and *Cleome viscosa* of the Cleomoideae exhibit the same chromosome number. This and other irregularitites would make it very difficult to accept the primitivity of the arboreal Capparidioideae. At any rate the evolution of these genera seems to have followed irregular lines.

III. Discussion

a. *Capparis zeylanica*—a secondary polyploid

On the basis of maximum association the gametic constitution of the species may be represented by:

aaaa
bbbb
cccc
ddd
ee
ff
g

It has already been indicated in the previous papers that seven is likely to be the primary basic number of the family and this would appear to be corroborated by the observations recorded herein. A natural cross between two seven-chromosomed parents (one of which had presumably its chromosomes changed structurally by gene mutation, etc.), would lead to the ultimate formation of a tetraploid by amphidiploidy with the somatic constitution $aaa'a'$–$ggg'g'$, on the basis that the parental genomes that

entered into the cross were represented by a–g × a′–g′. If the chromosomes a′–f′ undergo reduplication then we get a form with 2n = 40. The same result could also be obtained if the original seven-chromosomed ancestor (a–g) had been fertilized by the diploid gamete of the a′–g′ sister plant and if this were followed by syndiploidy it is likely that a forty-chromosomed plant might have survived by the deletion of a pair of chromosomes through meiotic or mitotic aberration. On this assumption the gametic genom of the resulting forty-chromosomed species may be represented as:

a a′a′
b b′b′
c c′c′
d d′d′
e e′e′
f f′f′
g′g′ or gg′

As evidenced by secondary pairing which is a strong indication of the ancestral homology between chromosomes, a and a′, b and b′ etc., we should expect a maximum association of seven consisting of six threes and one two (6(3) and 1(2)). But such an association is not to be found and groups of four are very common. This makes one infer that structural changes have played a part in the evolution of the species in addition to polyploidy; this can be explained by structural changes chiefly in the nature of reciprocal translocation as having taken place between different chromosomes. The expected somatic constitution may be represented as:

aa a′a′a′a′
bb b′b′b′b′
cc c′c′c′c′
dd d′d′d′d′
ee e′e′e′e′
ff f′f′f′f′
g′g′g′g′ or gg g′g′.

If segmental interchange takes place between a and e, b and f, and g and c chromosomes, then the result will be:

a(ae) a′a′ a′a′
b(bf) b′b′ b′b′
c(cg) c′c′ c′c′
d d d′d′ d′d′
e(ea) e′e′ e′e′
f(fb) f′f′ f′f′
g(gc) g′g′

On account of the new structural homology thus introduced, naturally bivalents a(ae) and e(ea) will be secondarily associated. Similarly b(bf) and f(fb) and so on. The result of this would be three groups of four, one group of three and two groups of two and a single bivalent unassociated. This means that the affinity between a(ae) and a′a′ bivalents has not been impaired by this structural change. The frequent association of bivalents of dissimilar size is a clear indication of an affinity between non-homologous chromosomes which have some segments in common.

As an alternative the following method of origin of the forty-chromosomed species may be considered:

Eight of the chromosomes of the tetraploid may be lost through deletion. Supposing the constitution of this 20-type be X in which D–G chromosomes have been lost, gene mutation or structural change may modify this type to produce a species of the constitution X′, in which corresponding D_2–G_2 chromosomes must be assumed to be absent. Amphidiploidy is likely to occur when X and X′ are crossed, so that a new type with 40 chromosomes arises. But here the maximum association should be 3(4) and 4(2), assuming, as we did, the deletion of the d′–g′ chromosomes. But instead we get 3(4), 1(3), 2(2) and 1(1). This can only be explained by reciprocal translocation as having taken place. Supposing the parential gametic genoms were of the following constitution:

X′			X	
A_2A_2	A_3A_3		AA	A_1A_1
B_2B_2	B_3B_3		BB	B_1B_1
C_2C_2	C_3C_3		CC	C_1C_1
	D_3D_3	×		D_1D_1
	E_3E_3			E_1E_1
	F_3F_3			F_1F_1
	G_3G_3			G_1G_1
D_2-G_2 deleted			D–G chromosomes deleted	

A cross between these would result in a species having the following constitution:

AA	A_1A_1	A_2A_2	A_3A_3
BB	B_1B_1	B_2B_2	B_3B_3
CC	C_1C_1	C_2C_2	C_3C_3
		D_1D_1	D_3D_3
		E_1E_1	E_3E_3
		F_1F_1	F_3F_3
		G_1G_1	G_3G_3

On this basis one would expect a maximum association of 3(4) and 4(2). If, however, reciprocal translocation takes place between D_1 and E_1 chromosomes, then we get, $D_1(D_1E_1)$ and E_1 (E_1D_1) and on the basis of homology between D_1 and D_3 chromosomes which we have assumed, we may get a group of three: D_3D_3, $D_1(D_1E_1)$, $E_1(E_1D_1)$. E_3E_3 would be left alone. F_1 and F_3, G_1 and G_3 will form two groups of two.

We find therefore that on either of these assumptions structural changes, chiefly reciprocal translocation would appear to have played a prominent part in the evolution of the species. In the former almost all the chromosomes were involved except the D chromosomes. In the latter, only a few chromosomes, D_1 and E_1 chromosomes would appear to have been affected structurally. Since, however, it is very common we find in groups of four, association of bivalents of dissimilar size, it is likely that it is these a-chromosomes that have undergone structural changes and as such the first assumption is more tenable.

b. Secondary association; its limitation as a guide to polyploidy

Evidence from secondary association alone cannot be regarded as conclusive in respect of ancestral homology and consequently of

the basic number. One of the most common factors which would make secondary pairing unreliable unaided by other evidence, is that structural changes of chromosomes may hamper this phenomenon. Structural changes of the homologous chromosomes may have taken place to a great extent in polyploids and as a consequence the degree of affinity required to cause attraction may not be present. Or translocations, simple or reciprocal, which are very common factors in the evolution of new species may give rise to higher associations, so that the basic number inferred from observed secondary paring may be erroneous. Numerical differences cause changes in frequency; high numbers of choromosomes tend to reduce the chance of association between similar chromosomes. Large size of the chromosomes appears to inhibit secondary pairing since it is seen generally only in organisms with small chromosomes.

In the present paper we have explained the maximum association on the basis of segmental interchange. Unaided by any previous knowledge it may not be proper to conclude that the maximum grouping represented the basic number. But since chromosome behaviour in two other genera, representative of the two subfamilies has already been studied and evidence let in to show that seven was likely to be the primary basic number of the family, the maximum association seen in this important genus has been interpreted in the way it has been done. The noteworthy fact is that reciprocal translocation and other structural changes which have undoubtedly played an important role in the evolution of the species have taken place in such a manner as to keep up the original number, though the groupings have undergone corresponding changes.

If chromosome interchange had taken place, then the absence of ring formation is rather hard to explain. But since the chromosomes are very small segmental homology manifests itself in an association of dissimilar chromosomes rather than in actual ring formation. Moreover it is not absolutely necessary that reciprocal translocation should be always followed by ring formation. A few cases have been reported where this has failed to occur. For instance, Clarke and Anderson (1935) have shown that in maize chromosome interchange takes place without the external evidence of ring formation.

Heilborn (1936) considers that no credence should be placed on secondary association as indicative of ancestral homology and that it is a purely physical phenomenon, that chromosomes of equal size are associated or tend to be associated irrespective of their homology. According to him it is not a specific attraction or pairing between homologous parts of chromosomes but the parallellism of the asso-

ciated chromosomes is mechanically induced through the polarity of the nuclei. Flowik (1938) has shown how this assumption is untenable so far as the genus *Carex* is concerned. In this paper additional evidence is to be found for not accepting the hypothesis. There is clear evidence of bivalents of different sizes associating. Primarily a result of ancestral homology, structural changes have also played a prominent part in this. There is also evidence of pairing of chromosomes of dissimilar size as could be seen from side views of metaphases. Observations of a similar nature have been made in other genera also. For instance in *Cicer* Iyenger (1939) has recorded the association between a short rod bivalent and a long one. Richharia (1937) has made a similar observation in the genus *Brassica*. These indicate that association is not determined by similarity in size, but rather by homology, even though his may mean an association of different sized bivalents.

c. General considerations

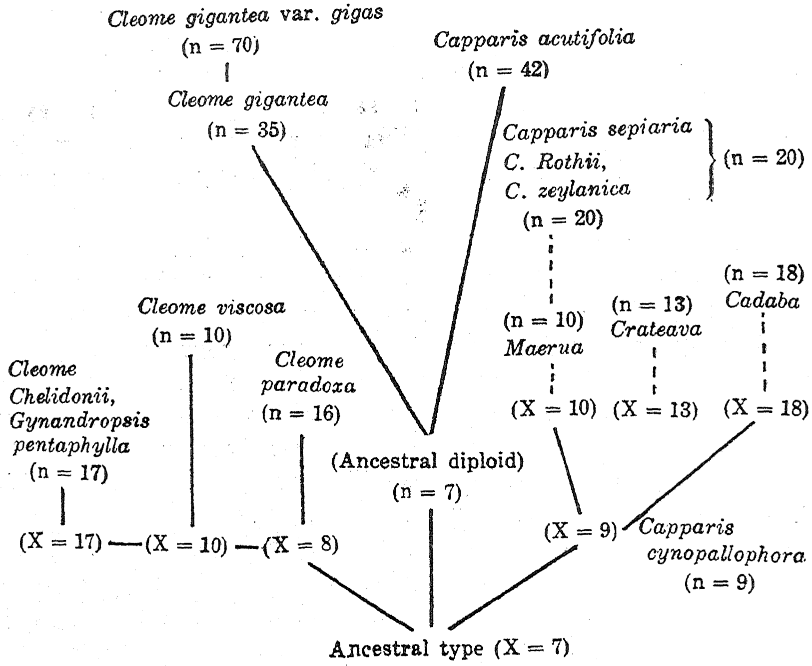
The chromosome numbers of the genera and the species so far investigated in the Capparidaceae form an irregular series. Polyploidy, structural changes, meiotic and mitotic aberrations have undoubtedly played an important part in the evolution of the species. The numbers known so far are so few that generalizations at this stage may not be quite warranted. A few remarks can, however be made regarding the distribution of the chromosome numbers in the various genera. The Capparidaceae are almost entirely tropical, a few subtropical and rarely temperate in their distribution. From the chromosome list now available one finds that generally speaking, the subtropical and temperate genera have greater chromosome numbers than the tropical ones. This is in keeping with observations made previously in a few families. For instances, in the Cactaceae, Stockwell (1935) found that the *Opuntiae* which were the most northern of the *Cacti* had higher chromosome numbers than the rest. Even among the same genus the more northern species had a greater chromosome number than the southern ones. Similarly Hagerup (1928) observes "it is worth noting that among these four pairs of species (including *Empetrum nigrum* and *E. hermaphroditum*) those with the higher chromosome numbers are always the ones growing farther north."

It is also likely that the original home of the Capparidaceae was the tropics. There would appear to be a correlation between distribution and polyploidy and it is quite conceivable that some of the subtropical species like *Cleome gigantea, C. gig.* var. *gigas, Cleome spinosa* etc., have essentially by polyploidy conquered new territories.

Navashin (1929) says,...."through changes in the rate of development a polyploid individual may acquire the ability of withstanding different climatic conditions and as a consequence penetrate into new territory." Hagerup (1933) also says, "polyploid forms may be ecologically changed so as to grow in other climates and formations where the diploid forms will not thrive." Clearly the species mentioned above are polyploids.

From purely cytological evidence—and even that is very meagre in this family—it is difficult to assemble the genera phylogenetically, not only because chromosome numbers are not absolutely diagnostic but also the numbers known so far are very irregular. But a few general remarks may not be out of place, none the less. There is to be seen, though not very apparent, an increase in chromosome numbers as one passes from the taxonomically more primitive to the more advanced Capparidaceae. Whether this is accompanied by any marked decrease in chromosome size, as it usually is the case, cannot be said. The chromosome numbers known uptodate, more or less confirms the taxonomic evidence that the genus *Capparis* is comparatively primitive. It must be said that it is difficult to determine the exact relations of the more primitive genera with one another. The cytological data available may, however, be utilized for indicating the broad sectional relations rather than for the alignment of species.

It has already been suggested that seven is likely to be the primary basic number of the family and from this, a number of secondary basic numbers have arisen and the various genera represent different balances of these numbers. In this, allopolyploidy as indicated by secondary association has played a prominent part. A species of *Capparis* shows the lowest chromosome number known in the family (2n=18) and conjoint with evidence available on morphological and taxonomical grounds, *Capparis* as representative of the subfamily Cappridioideae is to be regarded as more primitive. We find, however, that almost the same secondary basic numbers are to be found in the two subfamilies, Cleomoideae and the Capparidioideae and as such the evolution of the genera in the two tribes may be regarded as representing parallelism at least so far as the chromosome numbers are concerned. This may be represented diagrammatically in a rough manner as follows:



IV. Summary

The haploid chromosome numbers of the following have been determined for the first time:—*Capparis zeylanica* Linn. = 20. *Cadaba indica* Lamk.=18. *Maerua arenaria* Hook. f & Thomp.=10.

Secondary association is reported in *Capparis zeylanica* and the previous finding that the primary basic number of the family 7 is supported by observations made herein.

Evidence is found to show that polyploidy as well as structural changes of chromosomes have played an important part in the evolution of the species. The association of 4 bivalents is interpreted on this basis.

Secondary association and its limitations as the sole factor in determining ancestral homology are discussed in the light of the present findings and of the data gathered previously on other genera of this family.

Some general remarks are made on the distribution of chromosome numbers in this family and a tentative scheme formulated to show the phylogenetic evolution of some of the important genera.

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Rhythmic Banding in Protoplasm

By
Masa Uraguchi

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Introduction

In the course of some work on the effects of heavy metals on protoplasm (Seifriz and Uraguchi, 1941), I had the opportunity to study striking pictures of rhythmic coagulation in slime mold protoplasm.

Rhythmic zone formation or periodic precipitation in protoplasm and in tissues has been observed by a number of investigators. Lloyd (1928) has reviewed the subject. Lloyd and Moravek (1928) have compared the artificially produced banded precipitates in trichomes with those in gelatin in capillaries. An extensive study of rhythmic zone formation in plant tissues has been made by Küster (1913 and 1931). Balbach (1936) observed an alternation of granular plasma and hyaloplasm encircling particles of platinum black sprinkled on the plasmodia of slime mold. Weide (1939) saw similar zone formation in phycomycete plasmodia. Seifriz (1939) regards the formation of strata in slime mold plasmodia as a type of cytoplasmic coagulation, bearing a very obvious resemblance to Liesegang bands.

Observations

The material used in this work was the plasmodium of the slime mold, *Physarum polycephalum*. Stock cultures were kept growing on moist filter paper, and fed powdered oatmeal. For experimental study, small portions of a plasmodium were transferred to cover slips which had been coated with thin films of agar. These subcultures were kept in a moist chamber for several hours, time enough for the protoplasm to spread on the surface of the agar. The small plasmodia thus prepared were bathed in the following salt solutions, or the solutions were injected into the plasmodia by means of micropipettes.

The precipitation of rhythmic zones was observed on the application of all of the following solutions to slime molds, but in the case of none among numerous others tried. No buffer mixture, hydroxide, or acid at a pH above 3 produced rhythmic precipitates.

Salts	pH	Concentrations in molarity
$Cu(NO_3)_2$	above 4.5	0.01, 0.0005
$Cd(NO_3)_2$	,, ,,	0.1, 0.05, 0.01, 0.0005
$Pb(NO_3)_2$	,, ,,	0.1, 0.05
$Fe(NO_3)_3$	below 3	0.05
$UO_2(NO_3)_2$	,, ,,	0.1, 0.05
$Hg(NO_3)_2$	,, ,,	0.05

Acids	pH
HNO_3	1.4, 1.8, 2.2
HCl	1.4, 2.0
Buffer	2.8, 2.4

On bathing transferred plasmodia in the effective solutions, rhythmic zones begin to appear in scattered areas. There is first formed a densely granular center surrounded by a narrow hyaline, or less granular zone (Figs. 1, 2, 5, and 8). There then appears a dark granular band followed by alternating zones of hyaline and granular protoplasm extending centrifugally.

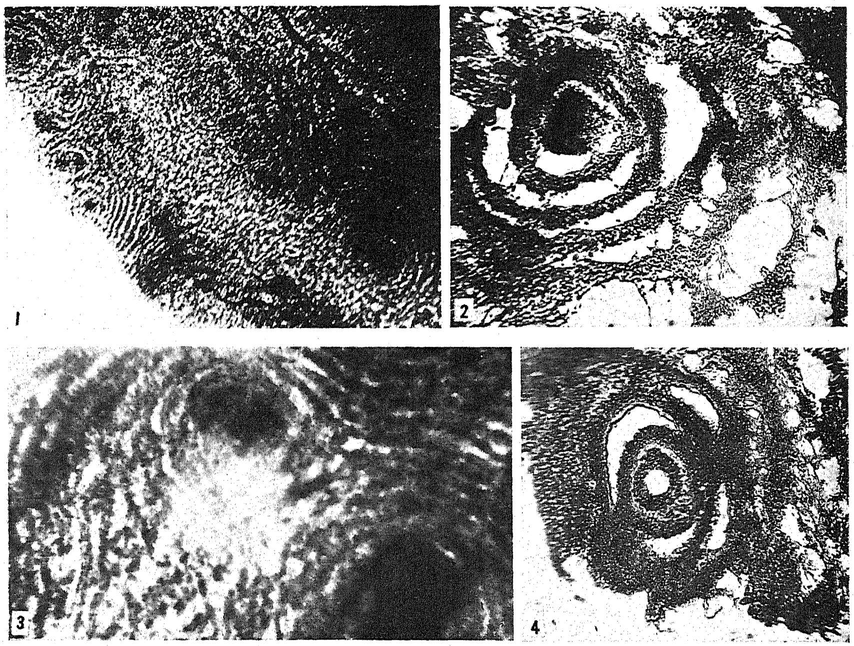

Fig. 1. Plasmodium bathed in dilute nitric acid: (pH 1.4). (×110). Fig. 2. Plasmodium bathed in 0.1 molar $Cd(NO_3)_2$. Fig. 3. Plasmodium bathed in 0.0005 molar $Cu(NO_3)_2$. (×730). Fig. 4. Plasmodium bathed in 0.1 molar $Cd(NO_3)_2$. (The coagulated core of the system has been washed away during handling.) (×110).

Quite a number of banded "islands" are formed within a plasmodium (Fig. 1). As each island enlarges the active streaming protoplasm is pushed back. The protoplasm within the banded areas is coagulated. The borderline between coagulated plasma and normal streaming plasma is usually clearly marked (Figs. 6, 9, and 11).

The rapidity with which alternating bands are formed, and their breadth vary considerably, depending on the nature of the solution, its concentration, and on the condition of the plasmodium. Thus, the zones formed in $Cd(NO_3)_2$ solutions of 0.1 to 0.01 molarity are much broader than those in any other solution.

After death caused by any of the foregoing salts, or acids, a plasmodium may be covered with ring systems (Fig. 1). Occasionally, two centers independently started became enclosed by a common system (Figs. 1, at left, and 3).

In some instances, as in $Cd(NO_3)_2$ solutions of 0.1 molar and 0.05 molar concentration, two neighboring granular bands separate after the completion of the ring system and in so doing stretch the intervening hyaline zone which then tears (Figs. 2, 4, and 5) forming thin thread-like strands between the two granular zones (Figs. 2 and 5).

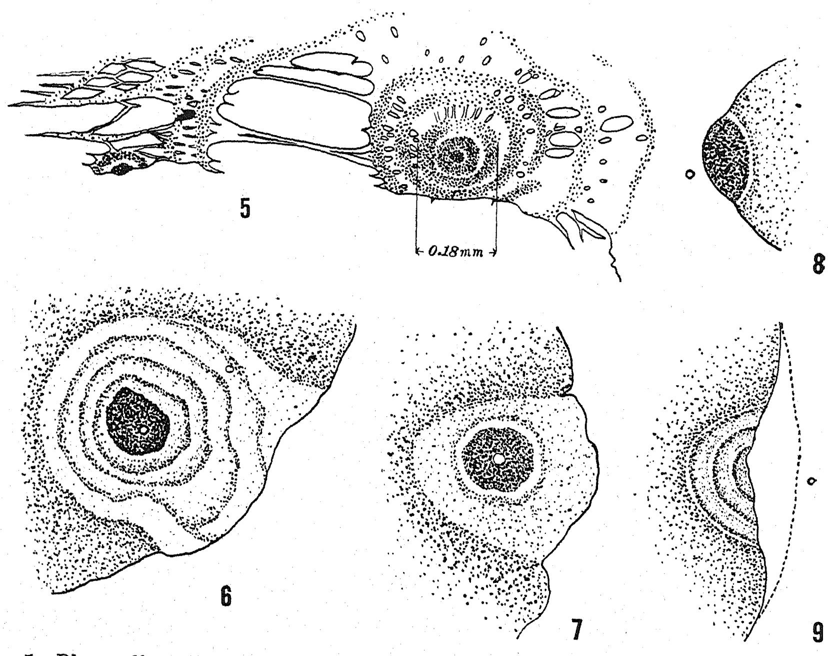

Fig. 5. Plasmodium bathed in 0.05 molar $Cd(NO_3)_2$. (×110). Fig. 6. Plasmodium injected with 0.1 molar $UO_2(NO_3)_2$. Fig. 7. Plasmodium injected with 0.05 molar $UO_2(NO_3)_2$. Fig. 8. 0.05 molar $UO_2(NO_3)_2$ applied externally with a micropipette. Fig. 9. 0.01 molar $Cu(NO_3)_2$ applied externally with a micropipette.

The foregoing phenomena all take place in plasmodia bathed in solutions. Similar zone formation occurs when salts are injected into plasmodia with the aid of a micropipette mechanically controlled in a micromanipulator (Seifriz 1936). When a salt solution is injected into a plasmodium, the protoplasm surrounding the tip of

the pipette coagulates immediately. Several layers of concentric rings then appear centrifugally around the dark coagullum (Fig. 6). Occasionally when a solution is injected, further concentric bands are not formed after the initial precipitation of a coagulum around the tip of the pipette, followed by a single narrow hyaline zone (Fig. 7), which ends the process. It would, therefore, appear that there are conditions other than specific salts and acids of definite concentrations for the production of concentric bands. This has been shown to be true for the formation of Liesegang rings in gelatine (Küster 1931, and Lloyd and Moravek 1928 and 1931). What these conditions are cannot be said. They are included in the "physiological state" of protoplasm. In non-living systems temperature is a factor.

When banded precipitation is produced by the injection of solutions, the alternating zones are formed only at the point of injection (Fig. 6), whereas in bathing many scattered centers appear, all forming concentric rings simultaneously (Fig. 1). The widespread formation of many separate islands of banded precipitates can be prevented even when the solution is externally applied if instead of complete immersion, the salt is concentrated at a restricted local area by means of a pipette. Such an application results in a single, superficial, semicircular banded precipitate (Figs. 8 and 9).

Discussion

Certain salts and acids applied to slime mold plasmodia, either by immersion or injection, react with the proteins of the protoplasm in such a way as to produce periodic precipitation which results in the formation of concentric rings around the center of diffusion. The hydrogen ion and the ions of heavy metals bring about this banded precipitation of the proteins in protoplasm. In the case of salts, such as $Fe(NO_3)_3$, $Hg(NO_3)_2$, and $UO_2(NO_3)_2$, which greatly lower the pH of their solutions, the H^+ ion is probably as much responsible as the metal ion, if not more so. In the case of $Cd(NO_3)_2$, $Cu(NO_3)_2$, and $Pb(NO_3)_2$, the pH of each of which is above 4.5, it is undoubtedly the metal alone which is responsible.

The banded precipitation of the proteins in protoplasm by acids and heavy metals is an example of the Liesegang phenomenon. The method of formation and the general appearance of the final picture are the same in the living material as in a non-living gel; thus, the breadth of the hyaline zones usually increases, and the degree of aggregation of the granules, or depth of color of the granulated

zones decreases, as the distance from the center increases (Fig. 10), which is characteristic of Liesegang phenomena.

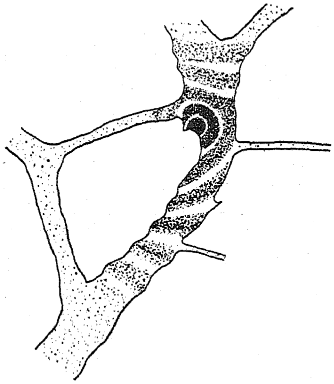

Fig. 10. Plasmodium bathed in 0.01 molar $Cd(NO_3)_2$.

The presence of scattered islands of banded precipitates surrounded by living and actively streaming protoplasm suggests that the protoplasmic surface is not uniform in composition or structure. When a plasmodium is immersed, the surrounding solution is in full contact with the entire surface, yet the dissolved substances enter only at certain isolated points around which the concentric bands are rhythmically precipitated. The permeability and composition of the membrane are therefore not uniform.

The precipitation patterns are in the main rather uniform as to type. However, sometimes the symmetry of the pattern differs considerably from the usual. Fig. 11 shows one of the irregular formations where a net structure has been formed.

Other irregularities occur which, however, are true to type but appear irregular because they are formed in restricted areas. When rhythmic precipitates are formed in strands, space limitations prevent development of the complete pattern (Figs. 10 and 12).

The periodic precipitations reported by Lloyd and Moravek (1928) in which the reagents meet in a gelatin layer formed in the capillary space between two glass plates, are closely similar to the banded patterns formed in slime mold plasmodia when bathed in the salts of heavy metals or in acids.

11

12

Fig. 11. Plasmodium bathed in 0.0025 molar $Cd(NO_3)_2$. (×110).
Fig. 12. Plasmodium bathed in 0.01 molar $Cu(NO_3)_2$. (×110).

Summary

1. The bathing of the protoplasm of slime molds in various salts and acids, or the injection of these into protoplasm, brings about

the formation of rhythmic bands through the precipitation of protoplasmic proteins.

2. Both the hydrogen ion and the ions of heavy metals produce banded precipitates in protoplasm.

3. The periodic precipitation of concentric rings in protoplasm bears a striking resemblance to the Liesegang phenomenon.

4. The more rapid entrance of solutes at certain restricted and isolated points of the plasmodium indicates pronounced differentiation in the permeability of the surface.

Acknowledgment

I wish to express my deepest appreciation to Professor William Seifriz for his critical supervision and unfailing encouragement.

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Intergeneric Hybridization in Cichorieae, V. Variation in karyotypes and fertility of *Crepidiastrixeris denticulato-platyphylla* [1]

By

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(With 25 figures in the text)

Received September 15, 1940

Introduction

By karyological and genetical studies *Crepidiastrixeris denticulato-platyphylla* Kitamura (=*Paraixeris denticulato-platyphylla* Nakai) was proved to be a natural hybrid of *Crepidiastrum platyphyllum* Kitamura (=*Crepidiastrum lanceolatum* var. *latifolium* Nakai) and *Paraixeris denticulata* Nakai (Ono and Satô, 1935), as Makino (1917) formerly suggested from the view point of taxonomy. A number of individuals collected at Tyôzyagasaki near Hayama in Kanagawa Prefecture were all found to have the same karyotype which was identical with that of artificially raised F_1 hybrids (Ono, 1937b). It was suggested that these individuals were either F_1 hybrids that were naturally produced and grew there last autumn, or karyologically balanced individuals which were propagated most abundantly after a long run of the struggle for existence. It was also suggested that the karyotypes of the individuals collected in other localities must be analysed in order to give fuller illustrations on the idiogram of the present species. For this purpose, the writer has studied those individuals of *C. denticulato-platyphylla* collected at Aburatubo near Misaki, Miura Peninsula, Kanagawa Prefecture. These individuals showed some karyological dissimilarities to each other. So the individuals from Tyôzyagasaki were only one type of this species. This suggests that this species may include many other types which differ both in karyological and morphological features.

In connection with this karyological study, a survey of the fertility of this species was carried out. Makino (1917) pointed out that the number of florets of this species is intermediate between that of *P. denticulata* and *C. platyphyllum*, the former having 13 florets and the latter 5. By the present survey it was revealed that

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1) Contributions from the Divisions of Plant-Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 258.

also in this point there is a great diversity. The fertility was determined by the percentage of ripe achenes to the total number of florets. Somewhat different fertilities were shown by different individuals. For comparison fertilities of *P. denticulata* and *C. platyphyllum* were examined. But the relationship between the karyotype and the fertility was not made clear, owing chiefly to the scarcity of the material. The number of involucre scales was also counted. As the taxonomists have noted, this character is much more stable than the number of florets.

The division of pollen-mother cells was studied. But as only four individuals survived to full maturation, the chromosome pairing and the morphology of paired chromosomes were not closely observed.

Material and Methods

The material used for the present investigation was taken from *C. denticulato-platyphylla* collected at Aburatubo, Miura Peninsula. A part of the plants was collected in the precinct of the Marine Biological Station of the Tokyo Imperial University, and the rest was collected on both sides of the newly opened canal on the way from Aburatuboguti to Aburatubo.

Collection was carried out in November 1938, when the plants were in full bloom, and some of the florets had fallen and achenes had already ripened. As this species is a biennial herb, these plants (M38) were of second year growth when collected. These were used for the floret-count and fertility-determination. Besides these, some younger plants (M39) were collected which were transplanted in the outdoor experimental garden. In April of the following year (1939), root-tips of these plants were fixed for cytological investigation. Four of these plants bloomed in autumn 1939, while many plants died of a disease during the summer. In November 1939, flower-heads were collected, and the floret-count and fertility-determination were carried out. Younger flower-heads were fixed for the study of the meiotic divisions.

The determination of fertility was made by a direct comparison of the ripe achenes in a flower head with the total number of florets in it. Ripe flower-heads whose involucre yet enveloped the achenes were taken, and the number of the ripe achenes and the total number of ovules were counted. These values were averaged separately and the ratio of the former to the latter was considered to be the value which indicates the mean fertility of the individual. This value will not be identical with the pollen-fertility or ovule-fertility, and some physiological factors which determine the achene

production are not to be neglected. In some cases pollen fertility was determined by the direct calculations of good and abortive pollen grains in the aceto-carmine smear preparations.

Karyotypes were determined in the mitotic division of the root-tip cells. Root-tips were fixed in Navashin solution, dehydrated with both ethyl-alcohol and buthyl-alcohol, imbedded in paraffin, sectioned in the thickness of $10\mu\pm$.

Young flower-heads were first fixed by acetic acid-alcohol and then transferred to 100% alcohol to harden the pollen-mother-cells. Some were prepared as aceto-carmine smear preparations, and others transferred to buthyl-alcohol and imbedded in paraffin, cut in the thickness of $20\text{–}25\mu$.

In staining, Newton's gentian-violet was used throughout. All the drawings except Figs. 1, 2, 19 and 20 were made with the aid of Abbe's drawing apparatus in the magnification of 3000×.

Observations

1. *The number of florets in each head and the percentage of ripe achenes.* In the first place, the floret-count and fertility-determination were made in *C. platyphyllum*, *P. denticulata* and their F_1 hybrid (Table 1). *C. platyphyllum* has 5 florets per head and 96%

Table 1. The number of florets and ripe achenes per head and fertility in individual plants

Plant	Number of heads examined	Number of florets per head		Number of ripe achenes per head		Fertility $\frac{b}{a}\times 100\%$
		Breadth of variation	Mean value (a)	Breadth of variation	Mean value (b)	
M38- 1	80	10–11	10.9	6–11	8.8	80.7
- 2	145	10–11	10.4	8–11	9.5	91.3
- 3	75	11–12	11.4	7–9	8.3	72.8
- 4	75	8–9	8.8	3–9	6.5	73.9
- 5	98	11–12	11.4	3–10	7.0	61.4
- 6	95	7–8	7.1	1–4	2.2	30.9
- 7	80	10–11	10.9	3–8	6.5	59.6
- 8	35	10–11	10.7	unripen	—	—
- 9	83	10–11	10.9	4–11	8.7	79.8
-10	80	9–11	10.4	2–5	3.2	30.8
-11	30	9–10	9.7	unripen	—	—
-12	30	10–11	10.7	6–7	6.7	62.6
-13	50	12–13	12.2	6–11	8.6	70.5
-14	30	9–10	9.7	7–8	8.3	85.6
-15	30	10–11	10.5	5–6	6.0	57.1
-16	30	12–13	12.7	7–13	9.0	70.9
-17	30	11	11.0	7–11	8.8	80.0
-18	50	9–12	10.6	7–10	8.4	79.3
-19	30	12–13	13.0	0–9	3.6	27.7
-20	30	11–12	11.3	8–10	9.3	82.3

Table 1—(*continued*)

Plant	Number of heads examined	Number of florets per head		Number of ripe achenes per head		Fertility $\frac{a}{b}\times 100\%$
		Breadth of variation	Mean value (a)	Breadth of variation	Mean value (b)	
M38-21	30	12-13	12.7	11-13	12.3	96.9
-22	30	11	11.0	10-11	10.5	95.5
-23	30	11-13	12.1	0-7	4.0	33.1
-24	30	10	10.0	9-10	9.5	95.0
-25	50	13-15	14.2	7-11	9.6	67.6
-26	40	12-13	12.5	2-9	6.0	48.0
-27	30	12	12.0	10-11	10.6	88.8
-28	30	12-14	12.8	11-13	12.2	95.3
-29	30	12-13	12.3	11-13	12.1	98.4
-30	30	11	11.0	7-10	8.5	77.3
-31	30	10-11	10.7	10-11	10.6	99.0
-32	30	11-12	11.3	9-10	9.3	82.3
-33	30	9	9.0	3-5	4.2	45.6
-34	72	12-13	12.7	1-11	7.2	56.7
-35	84	11-12	11.6	3-11	7.2	62.1
-36	44	11	11.0	2-6	4.3	39.1
M39- 3	50	11-12	11.4	unripen	—	—
- 7	50	10-12	11.2	unripen	—	—
- 8	50	11-13	11.6	0	0	0
-15	50	7-8	7.8	0-3	2.1	26.9
P. denticulata (Misaki)	50	13-14	13.1	12-14	12.9	98.4
C. platyphyllum (Hayama)	50	5	5.0	4-5	4.8	96.0
F_1 hybrid (*P. denticulata* ♀)	50	9-10	9.1	0-2	0.3	3.3

of fertility, while *P. denticulata* has 12–14 florets and 98% of fertility. Most flower-heads of the F_1 hybrid have 9 florets, which is intermediate between those of its parents, but its fertility is only 3.3%, which shows a great deviation from its parents.

Secondly, calculation was made with *C. denticulato-platyphylla* collected in the blooming state. The variation of the number of florets per head and the variation of fertility are shown in Tables 1 and 2 and Fig. 1. The breadth of the variation of the floret number

Table 2. The relation between the fertility and floret number per head

Number of florets per head	Number of plants having corresponding numbers of florets	Mean numbers of ripe achenes classified by the number of florets	Mean fertility %
14	1	9.6	67.60
13	6	8.4	64.61
12	5	7.3	60.80
11	15	8.5	77.27
10	5	6.1	61.00
9	2	5.3	59.00
7	1	2.2	30.98

is 7 to 15, the mode being 11. And that of ripe achenes is 0 to 13. The minimum percentage of the number of ripe achenes to that of the whole of florets per head is 27.69%. The relation between the number of florets and the percentage of ripe achenes is illustrated in Table 2 and Fig. 1. The highest percentage of ripe achenes falls on the heads with 11 florets, and the percentage diminishes according to the increase and decrease of the number of florets. And then it increases as the number of florets approaches to 13 or 5, the former of which being the number of florets in *P. denticulata* and the latter that in *C. platyphyllum*.

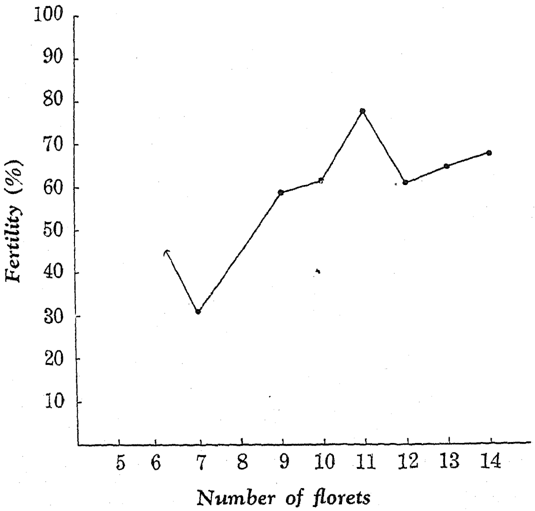


Fig. 1. The relation between the fertility and floret number per head (from Table 2).

Studies on those individuals whose karyotypes are known were expected to reveal something noteworthy as to the relation between the karyotypes and the number of florets or fertility. But the plants which bloomed in autumn 1939 were only four and further these were attacked by frost before the achenes fully ripened, so the results obtained were quite obscure, and no relationship was conclusively found therefrom (cf. Table 1).

2. *The number of involucre scales.* It is a well-established fact taxonomically that the number of involucre scales is more stable than that of florets. In connection with the floret-count and the fertility-determination above mentioned, the number of the involucre scales was calculated in the same material. In fact it was proved that it was remarkably stable. Hundreds of heads had to be examined in order to find one divergent head which had a different number of scales, nearly all the heads of an individual coinciding with the mode. In Table 3 are shown the results obtained where *C. platyphyllum* has 5 scales, *P. denticulata* 8 and their F_1 hybrid also 8. So it may be considered that the number of 8 scales is dominant over 5. *C. denticulato-platyphylla* has 6 to 8 scales. This seem to be an instance of the case where dominant genes are influenced by

Table 3. The number of involucre scales

Plant	Number of involucre scales
Paraixeris denticulata (Misaki) F_1 hybrid (*P. denticulata* × *C. platyphyllum*) *M38–17, –19, –20, –26, –27, –28, –29, –32, –33, –35, –36 *M39–7, M39–8	8
M38–16, –18, –22, –23, –24, –30, –31	7
M39–15	6
M39–3	6–5
Crepidiastrum platyphyllum (Misaki)	5

* M 38 and M 39 are *Crepidiastrixeris denticulato-platyphylla* collected at Misaki.

different compositions of karyotypes, dominance thus becoming incomplete. The number of involucre scales in regard to quantity-genes will be considered below.

3. *Karyotypes of Crepidiastrixeris denticulato-platyphylla collected at Aburatubo near Misaki.* As previously noted the karyotypes of this species is rather of a heterogeneous nature. Broadly speaking, the karyotype of this species is the combination of the chromosomes of *C. platyphyllum* and *P. denticulata* (Fig. 2). The plants collected at Tyôzyagasaki showed the karyotype quite similar to that of F_1 hybrids of these two species, the combination of haploid chromosomes of these parental species. But the present material showed rather a marked divergency in the karyotype.

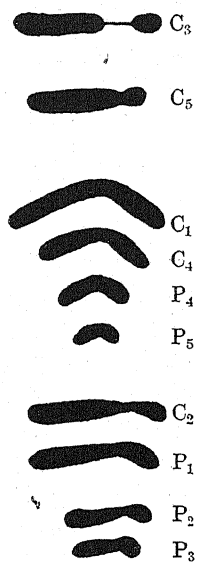


Fig. 2. The morphology of component chromosomes of F_1 hybrid *P. denticulata* × *C. platyphylla* which has both of the haploid chromosome sets of its parents (schematized).

The chromosome morphology of each individual is shown in Figs. 3–18. Chromosomes which are considered to be derived from *C. platyphyllum* are designated as C_1, C_2, C_3, C_4 and C_5, and those from *P. denticulata* as P_1, P_2, P_3, P_4 and P_5. The suffix figures 1, 2, 3, 4 and 5 are adopted only by the external morphology, chiefly the length of chromosomes: no considerations on the homology of chromcsomes are taken into account. The most characteristic is C_3 which has an extraordinarily long constriction. This chromosome can be identified most easily. C_5 is the unique i-shaped chromosome which has a subterminal constriction. This too is easily identified. There are 4 types of v-shaped and 4 types of

j-shaped chromosomes. Out of four v-chromosomes, C_1 is the longest and P_5 the shortest. These are easily identified. But to identify the intermediate two (C_4 and P_4) is rather difficult, C_4 being a little longer than P_4. Four j-shaped chromosomes are classified into two groups, the longer and the shorter. To the longer group belong P_1 and C_2. To distinguish these two is very difficult. These are nearly of the same shape and size except that P_1 is a little more medianly constricted. The shorter two, P_2 and P_3, are rather easily identified, P_2 being a little longer than P_3 (cf. Fig. 2.).

M 39–1. This plant is the unique monosomic plant ever found. In the mitotic figures are seen 9 chromosomes (Fig. 3). The missing chromosome seems to be P_2. The other components can easily be detected. Unfortunately, this plant died of a disease in summer 1939 and no further examination was possible.

M 39–2. This plant has 10 chromosomes (Fig. 4). C_3 and P_5 are easily detected, but C_1 and C_5 are missing. In place of these missing chromosomes are found C_2 and P_1 in pairs. So 4 chromosomes are derived from *C. platyphyllum* and the remaining 6 from *P. denticulata.*

M 39–3. This plant also has 10 chromosomes (Fig. 5). But C_5 and P_5 are missing. P_4 and C_4 are found in excess. So 5 chromosomes were derived from each of the parental species.

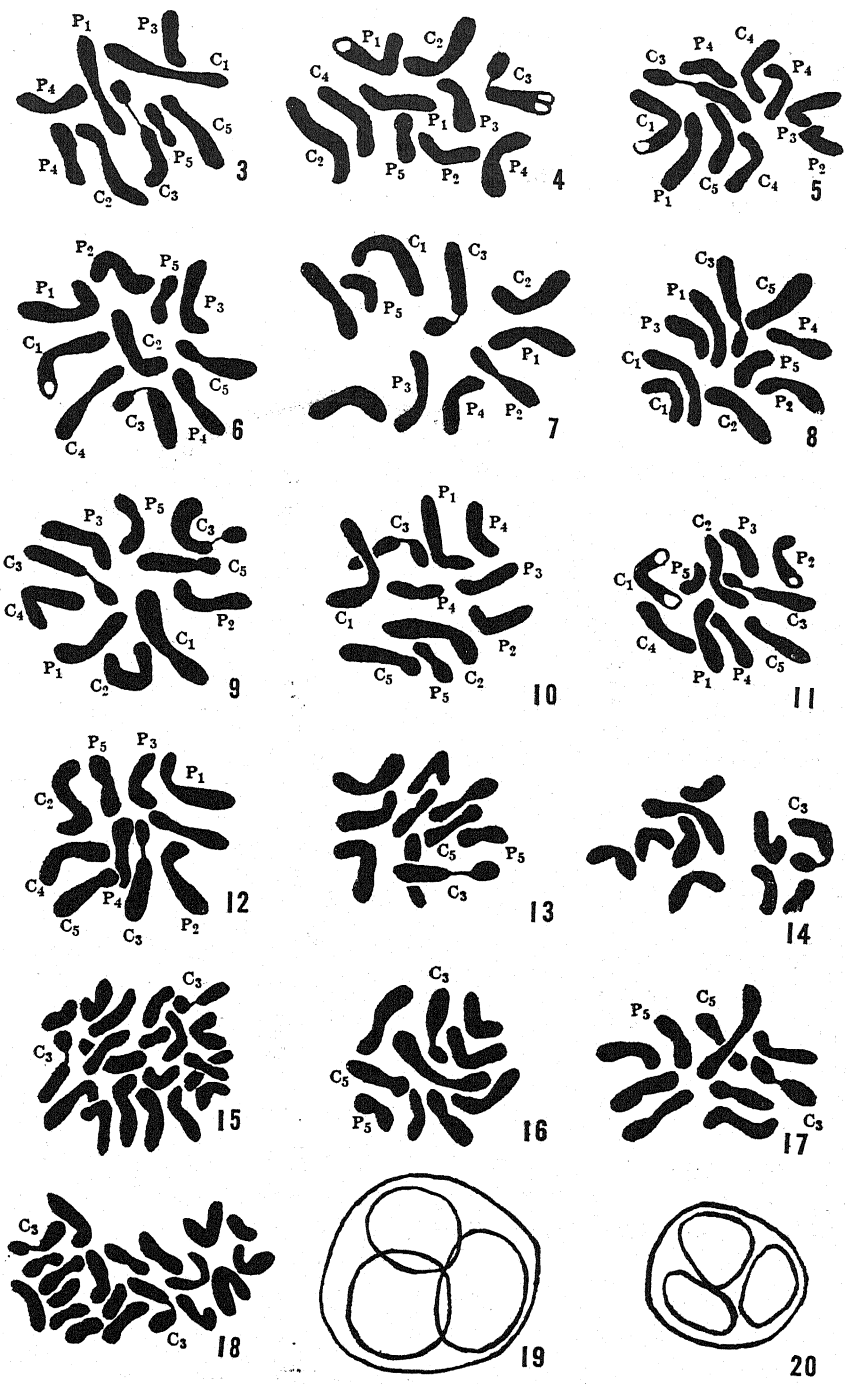
M 39–4, M 39–6, M 39–9. These plants have also 10 chromosomes, and the karyotype seems to be identical with the F_1 hybrid (Figs. 6, 8 and 11). The existence of the karyotype identical with the F_1 hybrid in these three plants seems to prove the prevalence of this type, a fact confirming the previous conclusion in the case of the plants collected at Tyôzyagasaki (Ono, 1937 b).

M 39–5. In this plant 10 chromosomes are also found (Fig. 7). But C_5 is missing, in the place of which, P_4 or C_4 is in excess. Owing to the scarcity of material, it was impossible to distinguish these two.

M 39–7. This plant has a pair of C_3 chromosomes which have an extraordinarily long constriction (Fig. 9). P_4 chromosome is missing instead. So it has 4 chromosomes of *P. denticulata* and 6 chromosomes of *C. platyphyllum.*

M 39–8. In this plant P_4 chromosome is in excess, but C_4 is missing (Fig. 10). So it has 6 chromosome of *P. denticulata* and 4 chromosomes of *C. platyphyllum.*

Figs. 3-18. Somatic chromosomes of *C. denticulato-platyphylla* collected at Misaki in 1938 (×3000). 3, M39-1. 4, M39-2. 5, M39-3. 6, M39-4. 7, M39-5. 8, M39-6. 9, M39-7. 10, M39-8. 11, M39-9. 12, M39-10. 13, M39-11. 14, M39-12 with diploid chromosomes. 15, M39-12 with tetraploid chromosomes. 16, M39-13.



17, M39-14. 18, M39-15. **Figs. 19–20.** Tetrads (×1200). 19, Diploid plant (M39-7). 20, Tetraploid plant (M39-15).

M 39–10. In this plant, C_1 is missing, and a submedian long chromosome is in excess (Fig. 12). It is not yet decided whether this is P_1 or C_2.

M 39–11, M 39–13, M 39–14. In these plants, C_3, C_5 and P_5 are seen (Figs. 13, 16 and 17). But the other components are not yet identified.

M 39–12. This is one of the noteworthy plants. Some roots have 2-ploid chromosomes and others 4-ploid (Figs. 14 and 15). The karyotype seems to be identical with that of the F_1 hybrid, though the components have not been fully identified.

M 39–15. This plant is also noteworthy. In root-tips, 20 or 4-ploid chromosomes are counted (Fig. 18). Owing to the larger number of chromosomes, each component of the karyotype is not yet identified. The habitus of this plant is very much stouter than the others (Fig. 21). So the entire plant seems to be tetraploid. The karyotype of this plant was not decided exactly, but it may be suggested that it is a modification of amphidiploid of the parental species, as two C_3 chromosomes were observed. The origin of this plant and M 39–12 will be discussed below.

Fig. 21. A branch of a tetraploid *C. denticulato-platyphylla* (M39-15).

4. *Meiotic divisions of Crepidiastrixeris denticulato-platyphylla*. Out of the 15 plants above noted, only 4 (M 39–3, –7, –8, –15) survived till the blooming stage, all the rest having decayed prematurely of the infection of a disease. This premature decaying was not caused by the genic or any such interior disturbance, but by the climatic circumstances under which these plants grew, for these plants had originally grown under an exceedingly mild coastal

climate and were transplanted into the experimental garden under a rather severe climate.

The pollen mother cells of these 4 plants which bloomed in autumn 1939 were examined karyologically. In this paper the results of preliminary observations only are given concerning the pairing of chromosomes and the paired bivalents. In the same material, abortive pollens were calculated (Table 4).

M 39–3, M 39–7. In the diakinesis and metaphase, pairing of the chromosomes is quite regular. Anaphase separation of the chromosomes also proceeds normally. Abnormal pollens are observed as rarely as in *P. denticulata* (Table 4).

Table 4. Abortive pollen grains

Plants	Number of pollen grains examined	Number of abortive pollen grains	Abortive pollen grains %
Crepidiastrum platyphyllum (Hayama)	458	8	1.75
Paraixeris denticulata (Nakamura)	504	16	3.17
F_1 hybrid (*P. denticulata* × *C. platyphyllum*)	482	12	2.49
M39-3	470	16	3.40
M39-7	502	23	4.58
M39-8	568	92	16.20
M39-15	450	14	3.11

M 39–8. In this plant pairing had also taken place normally but the percentage of abortive pollens was 4 or 5 times larger than the former two (Table 4). In spite of this higher percentage of abnormal pollens, no failure of pairing was observed and the separation in anaphase seems to proceed normally in many cells.

M 39–15. This plant is tetraploid as noted above. Pollen-grains are somewhat larger than those of other diploid plants (cf. Figs. 19 and 20), and in its pollen mitosis 10 chromosomes are clearly counted (Fig. 22). The percentage of abnormal pollens is as small as in M 39–3 and M 39–7 (Table 4). In the diakinesis and metaphase, 5 elements, rarely 6 or more rarely 7 elements, of chromosomes are observed (Figs. 23–25). The 10 bivalents to be expected were not observed. In most cases 5 tetravalents were formed, and the occurrence of the 6 or 7 elements of chromosomes must have been the result of rare failure of tetravalent formation in some chromosomes.

The precise determination of the karyotype of this plant was not possible, but it may be a modification of the amphidiploid of

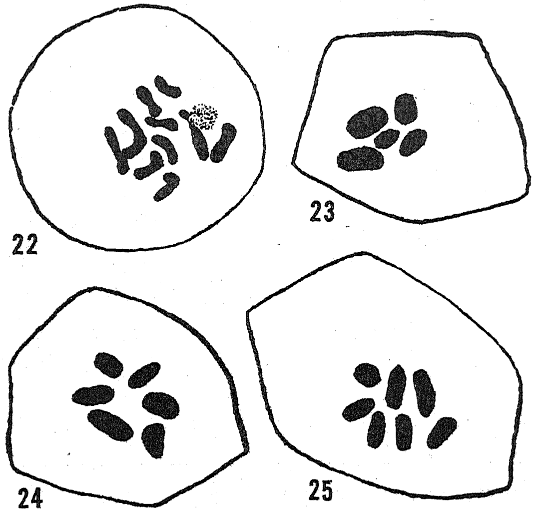


Fig. 22. Pollen mitosis of M39-15 (×3000). Figs. 23–25. Meiotic metaphases of M39-15 (×2400). 23. Pmc with 5 units of chromosomes. 24. Pmc with 6 units of chromosomes. 25. Pmc with 7 units of chromosomes.

P. denticulata and *C. platyphyllum*. It must be noted that there is a constant formation of tetravalents in spite of the heterogeneity in the morphology of chromosomes.

Discussion

In the preceding paper (Ono 1937 b), it was reported that the karyotypes of *Crepidiastrixeris denticulato-platyphylla* collected at Tyôzyagasaki are uniformly similar to that of F_1 bybrids of *Crepidiastrum platyphyllum* Kitamura and *Paraixeris denticulata* Nakai. But the present material collected at Aburatubo presents various karyotypes including trisomic and tetraploid forms as well as a plant with diploid-tetraploid chimera, of which, however, the tetraploids are supposed to be of secondary origin. Out of the 15 plants examined 3 had the karyotype identical with that of the F_1 hybrid. From the fact that F_2 progeny of *C. platyphyllum* and *P. denticulata* has various karyotypes, as reported previously (Ono, 1937 a), diversity in the karyotypes of a natural population may be reasonably understood. The existence of the karyotype of the F_1 hybrid in these 3 plants and in all of those examined in the previous case seems to imply that the plant with the same karyotype as F_1 hybrids has the highest fertility and is propagated most vigorously as noted before (Ono, 1937 b). Previous investigators who have dealt with natural hybrids have also reported the presence of many introgressive forms (Goodwin, 1937; Yenikeyer, 1938; Riley, 1939). Accordingly it may be concluded that in nature there exists a certain amount of natural hybrids, with many introgressive forms that do not belong to any one species.

The number of florets per head is somewhat varied with the individual (Table 1). The maximum frequency of plants classified by the mean number of florets falls on the plants with 11 florets and

the maximum of fertility also on those with 11 florets (Table 2, Fig. 1). It is only too natural that the individuals with the highest fertility are found most abundantly. The frequency and the fertility decrease with both the increase and decrease of the number of florets. But, as the parental species have 5 and 13 florets and their fertilities are very high, there are three extremes, 5, 11 and 13 in the floret-frequency and the fertility. In the intervals from 5 to 11, and 11 to 13, there have been found plants with rather low fertility. So the most balanced individual in nature is that with 11 florets. The number of florets of the F_1 hybrid is 8. Accordingly the most abundant forms in nature seems to present a slight morphological difference from the F_1 hybrid. The plants of which both the karyotype-determination and floret-count were carried out have been only 4 in number, so the relation between the karyotype and the number of florets is not yet decided. Whether the number of florets is influenced by a particular chromosome or by a set of chromosomes as a whole, remains to be determined by further experiments.

The number of involucre scales is of great stability and used as a taxonomic criterion in this group of plants. The number of scales is 8 in *P. denticulata*, 5 in *C. platyphyllum* and 8 in their F_1 hybrid. So 8 is dominant over 5. The number of scales of *C. denticulato-platyphylla* shows a variation from 6 to 8 (Table 3). Plants with 8 scales are more frequently observed. If this character is determined by a gene or genes on a single chromosome the number of scales must be either 5 or 8, according as a chromosome of *P. denticulata* with the gene or genes is absent, or there is found one or two. Unexpectedly, however, there were found, in this case, many plants with 7 involucre scales. This may be the result of the peculiarity of the quantity-genes. The polymeric nature of such genes was first pointed out by Nilsson-Ehle (1909), and many others have followed him (East, 1913, 1916; Sax, 1923; Warren, 1924; Rasmusson, 1933, 1935; Lindstrom, 1931; Green 1931, 1933; Wexelson, 1933; Smith, 1937; Sinnott, 1937; Barlels 1940; Watkins and Ellerton, 1940). In the case of the involucre character of *C. denticulato-platyphylla*, a number of polymeric genes seem to be concerned, and these genes act in an additive way. As the results of such an action of these genes, dominance becomes imperfect according to the chromosome complex.

A plant with diploid-tetraploid chimera and a tetraploid plant were found. On the origin of these plants, the following considerations may be made. These plants were perhaps diploid when growing on the coast of Aburatubo. If in nature tetraploid plants occur in such a high percentage, there must be found a considerable amount of

triploid offsprings in their neighbourhood. But no triploid has been found in the present and preceding experiments (Ono, 1937 b). It may be considered therefrom that these plants became tetraploid in the experimental garden. Considering the climatic conditions under which these plants have grown, this presumption is all the more strengthened. These plants must have germinated in spring 1938, and have grown under a favorable climate on the seashore up to the time of collection in autumn 1938. Then these plants were transplanted under a rather severe climate of Tokyo where the temperature often falls below –5 in winter. Thus they were often frozen totally and the plants M 39–12, –13, –14 and –15 have nearly died. In spring 1939, from a few remaining cells new buds and roots sprang forth. In course of such recovering of vitality, there must have been many chances for the chromosome doubling to occur. It is supposed that in M 39–15 the doubling of chromosomes occurred in some cells, and that it was only these cells that developed into the above mentioned plant, but that in M 39–12 chromosome doubling occurred in some cells only and developed at the same time with the diploid cells, thus forming a chimeral plant. If this presumption is correct, it affords an explanation that there are many polyploid plants in high mountains, polar regions as reported by many authors (Hagerup, 1931; Tischler, 1934, Flovik, 1938). The present author has undertaken a small experiment to test whether or not castration callus is easily formed in this plant. But the results were negative in spite of the application of heteroauxin-lanolin-pasture. Killing the outer cells by frost seems to be more favorable for this purpose than the total cutting off of stems.

M 39–15 is considered as an allotetraploid. In the meiotic divisions of this plant about 10 bivalents had been expected. But actual observations proved that in almost all of the pollen mother cells 5 tetravalents were formed. As has been previously noted pairing is quite regular in the F_1 hybrid of *C. platyphyllum* and *P. denticulata*, and as many chiasmata are formed as in its parents in spite of the morphological difference of the chromosomes (Ono and Satô, 1935). The tetravalents of the present plant may be analysed into two morphologically different pairs of two chromosomes. These two chromosomes in each pair must be totally homologous. The behavior of these four chromosomes in meiosis is expected to be that the two homologous chromosomes must pair with each other, but that the two resulting pairs need not unite themselves to form a tetravalent even if there is partial homology between them. But in this plant tetravalents were constantly formed. Thus it may be said that the pairing power of these chromosomes which pair constantly but differ

morphologically is as strong as that of totally homologous ones. So these heteromorphous chromosomes may be considered as homologous in spite of their morphological difference. The homology of chromosomes, therefore, cannot be determined merely by their morphological characters.

Summary

1) There are considerable variations in fertility and karyotypes of *Crepidiastrixeris denticulato-platyphylla* Kitamura collected at Aburatubo near Misaki (Fig. 3–18).

2) The number of florets and involucre scales per head was counted. The fertility was determined directly by the ratio of the number of ripe achenes to that of florets. The plants with 11 florets have the highest fertility (Table 2). These plants seem to be different in some way from the F_1 hybrids which have 9 florets.

3) The number of involucre scales is much more stable than the number of florets (Table 3). This character shows incomplete dominance and seems to be conditioned by the polymeric genes like many other quantity characters.

4) One hypoploid (monosomic) form (Fig. 3), one with diploid-tetraploid chimera (Figs. 14 and 15) and one tetraploid form (Figs. 18, 20 and 21). But these tetraploid plants seem to be of secondary occurrence after transplantation into the experimental garden from the mild climate of the coastal region.

5) Meiotic divisions of some plants were studied. Pairing and separation of chromosomes proceed fairly regularly. In the tetraploid plants also a regular formation of tetravalents was observed (Figs. 23–25).

The writer wishes to thank Prof. Y. Sinotô of the Tokyo Imperial University under whose direction and guidance these investigations have been undertaken, and also Prof. M. Eri of the Marine Biological Station of the Tokyo Imperial University, who facilitated him to collect the plants used in the present work.

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Über die Spindelfigur bei der somatischen Mitose der Prothalliumzellen von *Osmunda japonica* Thunb. *in vivo*[1]

Von

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(Mit 25 Textfiguren)

Eingegangen am 26. November 1940

Einleitung

Seitdem es STRASBURGER (1880) gelang, bei den Zellen der Staubfadenhaare von *Tradescantia virginica* die Kernteilung im lebenden Zustande durch alle Phasen zu verfolgen, gelten die *Tradescantia*-Haarzellen als unübertreffliches Material für die Untersuchung der Mitose *in vivo* (vgl. KÜSTER 1933). Jedoch haben wir jetzt festgestellt, daß diese Haarzellen als Material für die Lebendbeobachtung der achromatischen Figur nicht immer geeignet sind. Die Spindelfigur der Haarzellen tritt fast voll im Zellraum auf, und zwar ist sie selbst mit langen großen Chromosomen so angefüllt, daß ihrer äußeren Form die Deutlichkeit fehlt. Im Gegensatz zum schmalen Zellraum der *Tradescantia*-Haarzellen geht die somatische Mitose bei den Prothalliumzellen einiger Farnen in einem stark vakuolisierten großen Zellraum vor sich, und da kommt oft ein Teil der Spindelfigur unbedeckt von der Zytoplasmaschicht mit der Vakuole in Berührung, wodurch sich die äußere Form anschaulich erkennen läßt.

Wären zur Zeit STRASBURGERs alle Phasen der Mitose statt an den *Tradescantia*-Haarzellen an jungen Farnprothalliumzellen *in vivo* verfolgt worden, so würde die Kenntnis über die achromatische Figur etwas anders als die heutige sein. Man würde das Verschwinden der Kernwandung und das Vermischungsvermögen der Kernflüssigkeit mit dem Zytoplasma beim Auftreten der Metaphasespindel als Folge der Fixierungsartefakte und die Nicht-Mischbarkeit derselben als einen wirklichen Zustand der Spindelsubstanzen anerkennen.

In der vorliegenden Arbeit habe ich die morphologischen Veränderungen der achromatischen Figur, nämlich ihre Entstehung, ihre kontinuierliche Veränderung zum Phragmoplasten, die Ent-

1) Contributions from the Divisions of Plant-Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 266.

wicklung der Scheidewand und den Abbau der achromatischen Substanzen durch Lebendbeobachtung, studiert. Weiterhin bedürfen wir auf Grund der mitotischen Vorgänge *in vivo* und der Atraktoplasma-Theorie der Richtigstellung des bis heute fast ausschließlich auf fixierten Präparaten beruhenden Begriffes über die achromatische Figur.

Material und Methode

Die Mitose der Prothalliumzellen von folgenden Farnen wurde geprüft[1]: *Equisetum arvense* L. var. *boreale* RUPR., *Nephrolepis hirsutula* PRESL., *Polypodium Fauriei* CHRIST, *Pteris Fauriei* HIERON., *Osmunda japonica* THUNB. und *Osmunda bromeliaefolia* COPEL. Von diesen Arten eignen sich die Prothalliumzellen von *Osmunda japonica* THUNB. für die Lebendbeobachtung der Mitose am besten, da dieser Farn an Größe des Kern- und Zellraums und Zahl der mitotischen Figuren die anderen Arten übertrifft. Um die Lebendbeobachtung der Mitose der Prothalliumzellen zu erleichtern, ließ ich die Sporen im hängenden Tropfen einer Feuchtkammer keimen. Das Leitungswasser, welches für das Keimen der Sporen und für das Wachsen der Prothallien nötig ist, wurde in einer Reihe von schmal geschnittenen *Sphagnum*-Blättchen unter einem Deckglas der Feuchtkammer angesammelt und bildete also dort schmale hängende Wasserkanäle (Fig. 1). Ich stellte die Sporen vorher mit einer Mikronadel vorsichtig an eine Seite der Blättchenreihe und ließ sie dort zu Prothallien heranwachsen (Fig. 2).

Im hängenden Tropfen keimen die Sporen in einigen Tagen, und diese jungen 5-20zelligen Prothallien eignen sich für die Lebendbeobachtung der Mitose am besten. Bei noch weiter herangewachsenen Prothallien treten die Mitosen an der oberen Seite sehr selten, aber an ihrer unteren häufiger auf; diese Mitosen liegen aber fast immer außerhalb der Brennweite des Ölimmersionsobjektivs und sind für die Lebendbeobachtung nicht geeignet. Dasselbe gilt auch für sehr junge zwei- oder dreizellige Prothallien, da ihre dicht mit Chloroplasten angefüllten Zellen undurchsichtig sind. Das rauhe bräunliche Exospor der *Osmunda*-Sporen bleibt nach deren Keimen lange Zeit zurück, bedeckt gewöhnlich die Spitze der jungen Prothallien und wirkt oft störend auf die klaren Bilder der mitotischen Figuren. Daher beseitigte ich immer vorsichtig vor der Beobachtung bei drei- oder vierzelligen Prothallien mit einer Mikronadel das Exospor (Fig. 2).

1) Diese Pflanzen wurden mir von Herrn Dr. S. MOMOSE aus seinem Untersuchungsmaterial zur Verfügung gestellt. An dieser Stelle spreche ich ihm meinen verbindlichsten Dank aus.

Als optische Apparate habe ich LEITZ Ölimmersionsobjektiv 100× und Okular 10× und für die Mikrophotographierung LEITZ Makam mit einem Periplanokular 10× benutzt, wobei ich die elektrische Lampe nach FUJII als Lichtquelle verwendete. Diese Lampe besteht aus einer Doppel-Filamente-Birne (100 und 500 watt Filamente); man kann mittels des Anschalters augenblicklich das 100 watt Licht zum 500 watt Licht umschalten. Für die Lebendbeobachtung eignet sich das 100 watt Licht, genügt aber nicht für die Mikrophotographierung und es bedarf hier des 500 watt Lichtes. Von den Mikrophotographien im Text sind Fig. 16–21 und 23 in zweifacher Größe (Vergr. ca. 1800×) und die anderen in der Größe der originalen Negative (Vergr. ca. 900× mit Ausnahme von Fig. 1–2) reproduziert.

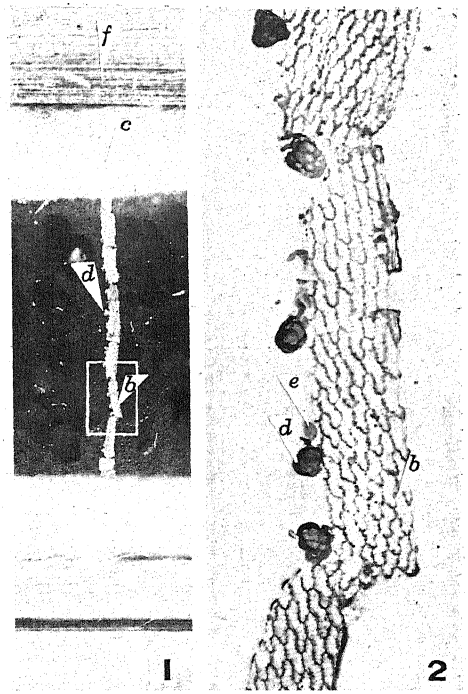

Fig. 1. Ein Teil der Feuchtkammer (Vergr. ca. 3×): *b* eine Reihe von schmal geschnittenen *Sphagnum*-Blättchen, *c* mit Wasser durchtränkte Wattestreifen, *f* ein Teil der Mittelleiste der Feuchtkammer, *d* das junge Prothallium, 10 Tage nach dem Aussäen. Fig. 2. Ein Teil der *Sphagnum*-Blättchenreihe (Vergr. ca. 30×): *e* das beseitigte Exospor.

Beobachtung

Karyokinese: Bei den Prothalliumzellen von *Osmunda japonica* sieht die Struktur des Ruhekernes granulär aus, wie der Ruhekern der *Tradescantia*-Zellen (Fig. 15, 22). Aber die Größe der *Osmunda*-Kerne beträgt ungefähr zwei Drittel der *Tradescantia*-Kerne. Am Anfang der Prothalliumentwicklung wachsen die Zellen so merklich, daß die noch teilungsfähigen Kerne in stark vakuolisierten großen Zellen liegen (Fig. 3). In solchen meristematischen Zellen gestaltet sich das Zytoplasma zu einen dünnen Plasmawandbelag, welcher fast gleichmäßig mit Chloroplasten bedeckt ist. Bei der Beobachtung der Karyokinese liegen jedoch diese Chloroplasten außerhalb der Brennweite des Ölimmersionsobjektivs, da die Brennweite im Ver-

gleich mit der Größe des Hohlraums um den Kern sehr gering ist.

In großen Zellen pflegt der in der Teilung begriffene Kern nicht im Zentrum des Zellraums sondern an einer Seite der Zelle zu liegen und dort die Spindelbildung durchzuführen (Fig. 3). Diese Stellung des Kernes zur Mitose, welche hauptsächlich durch ein eigenartiges Verhalten des Zytoplasmas um den Kern hervorgerufen wird, erleichtert die Entwicklung der Spindelfigur und die Bildung der Scheidewand. Im Gegensatz zu fixierten Präparaten ist das Verhalten dieses Zytoplasmas in lebenden Zellen auffallend. Mit dem Einsetzen der Karyokinese bewegen sich einige der Zytoplasmateile am Wandbelag zur Kernumgebung, einige andere jedoch in der entgegengesetzten Richtung; jedenfalls bewegen sich einige Chloroplasten vom Wandbelag zur Kernumgebung (Fig. 4). Während der Hin- und Herbewegung der Plasmastränge um den Kern, treten viele Plasmastränge an der der Zellwand näher liegenden Seite der Zelle auf und führen den Kern näher an die Zellwand. Infolge dieser zytoplasmatischen Vorbereitung findet die Karyokinese nicht im Zentrum des herangewachsenen großen Zellraums sondern immer an der Zellwand anliegend statt. Die Polplasmastränge, deren eigenartigen Charakter ich (1935) schon bei Mitosen der *Tradescantia*-Haarzellen erklärt habe, treten auch bei Prothalliumzellen auf und schließen sich der Bestimmung der Spindelachse und daher auch der Richtung der Scheidewand an (Fig. 7).

Betreffs der Lage des Prophasekernes wurde neulich von SINNOTT und BLOCH (1940) ein ganz entgegengesetztes Beispiel bei der Mitose der Wundgewebe einiger Pflanzen berichtet. Bei ihren Materialien bewegen sich die in der Teilung begriffenen Kerne vom Plasmawandbelag ins Zentrum der stark vakuolisierten Zelle. Um diese Kerne herum sammeln sich viele Zytoplasmastränge an und bilden eine, aus miteinander etwas verbundenen Zytoplasmasträngen bestehende Platte. Diese Platte, die sich in der Äquatorialebene des Zellraums verbreitet, bezeichneten SINNOTT und BLOCH als „Phragmosome“; diese Plasmaplatte nimmt die Stelle ein, an welcher später die Scheidewand gebildet wird.

Im Kernraum der *Osmunda*-Zellen entwickelt sich die granulär aussehende Chromonemastruktur zu Chromatinfäden und weiter zu Chromosomen (Fig. 3–5). In der späten Prophase wird der Kernraum in seiner Mitte mit den Chromosomen und in seiner Peripherie mit dem aus Karyolymphe entstandenen Atraktoplasma gefüllt. Das Atraktoplasma, welches sich allmählich an den entgegengesetzten polaren Seiten des Kernraums ansammelt, erweist sich als durchsichtig und granulenfrei, wie die Karyolymphe im Ruhekern, aber das Atraktoplasma verhält sich bei physikalischen oder chemischen

Angriffen ganz anders als die letztere, wie ich (1935, 1939, 1940) bereits diese Eigenschaften wiederholt durch experimentelle Untersuchungen festgestellt habe. Besondere Aufmerksamkeit widmete ich der Veränderung der Kernwandung um festzustellen, ob das Verschwinden der Kernmembran und infolgedessen eine direkte Vermischung der Kernflüssigkeit mit dem Zytoplasma bei dem Auftreten der Metaphasespindel zustandekommen oder nicht.

Am Ende der Prophase verändert der Kern seine äußere Form kontinuierlich von einer kugelförmigen (Fig. 4) zu einer elliptischen (Fig. 5) und weiter zu einer spindelförmigen und tritt in die Metaphase ein (Fig. 6). Die angeschwollenen Teile des Kernraums in Fig. 5 bilden in der folgenden Teilungsphase die Spindelpole; daher kann man während dieser Gestaltsveränderung ununterbrocken die Wandung der Teilungsfigur beobachten. Man stellt dabei fest, daß weder das Verschwinden der Kernwandung am Ende der Prophase noch ein Vermischen der Kernflüssigkeit mit dem Zytoplasma beim Auftreten der Metaphasespindel stattfindet. Dieses ist der echte Zustand der Metaphasespindel in lebenden Zellen. Aber es kommt nicht selten vor, daß die Wandung einer jungen Spindelfigur durch sich bewegende Zytoplasmateile oder durch eine Ansammlung von Chloroplasten an der Kernoberfläche teilweise unklar wird.

In der Metaphase sammelt sich an beiden Spindelpolen mehr Zytoplasma als an der Äquatorialgegend an (Fig. 6). Die Grenzoberfläche des Atraktosoms ist dort besonders deutlich erkennbar, wo sie mit dem Zellsaft in Berührung kommt. Es kommt dabei nicht in Frage, ob eine dünne Zytoplasmaschicht zwischen ihnen vorhanden ist oder nicht, da sich das Atraktoplasma selbst weder mit dem Zytoplasma noch mit dem Zellsaft im lebenden Zustand als mischbar erweist.

Im Gegensatz zur Klarheit des Atraktosoms bei *Osmunda*-Zellen sind die Chromosomen in ihm infolge ihrer Kleinheit und ihrer großen Anzahl schwer zu verfolgen. Den Untersuchungen von OKUNO (1936) nach, enthält die Zelle in der Wurzelspitze von *Osmunda japonica* THUNB, 44 Chromosomen; ihre Gestalten treten *in vivo* nicht so deutlich wie bei *Tradescantia*-Zellen hervor, jedoch kann man einige von ihnen im mikroskopischen Sehfeld erkennen und auf Grund ihrer Stellungen die Teilungsphasen feststellen.

In Fig. 7 setzt die Wanderung der Tochterchromosomen nach den Spindelpolen ein, und die Kernteilung tritt in die Anaphase ein. Fig. 8 weist eine späte Anaphase auf, in der sich die Tochterchromosomen von einander getrennt zur Spindelachse parallel ordnen. Wenn die Tochterchromosomen die Spindelpole erreichen, wird das Atraktoplasma gezwungen, sich an beiden polaren Teilen zur

Äquatorialgegend hin fortzubewegen (vgl. WADA 1935); dann fängt das Atraktosom in seiner Äquatorialgegend zu schwellen an und verändert sich allmählig zu einem tonnenförmigen Körper (Fig. 9). Dieser Körper ist nichts anderes als der Phragmoplast. Aus diesem Verhalten des Atraktosoms und auch aus seinen weiteren Veränderungen ergibt sich, daß der Phragmoplast stofflich vom Atraktoplasma und gestaltlich vom Atraktosom infolge kontinuierlicher Veränderungen gebildet wird (Fig. 8–10, 18–20). Die Nicht-Mischbarkeit des Atraktoplasmas mit dem Zytoplasma wird auch dem Phragmoplasten übermittelt und dauert einige Zeit an. Nach dem Auftreten des Phragmoplasten setzt fast gleichzeitig die Entstehung der Wandanlage ein und die Teilungsfigur tritt in die Telophase ein (Fig. 9).

Zytokinese: Im Gegensatz zur Zytokinese in einem schmalen Zellraum wie bei *Tradescantia*-Haarzellen scheint die Scheidewandbildung in einem stark vakuolisierten großen Zellraum bei *Osmunda*-Zellen sehr verwickelt zu sein. In Fig. 10 tritt die Mitose in die mittlere Telophase ein; das Zytoplasma, welches sich in der Meta- und Anaphase an den Spindelpolen stark anhäuft, bewegt sich jetzt vom Spindelpol zur Äquatorialgegend hin und bedeckt den Phragmoplasten. Da der Phragmoplast an der Mutterzellwand anliegend gebildet ist, kommt die noch sehr junge Zellplatte fast gleichzeitig mit dem einen Phragmoplastrand auf leichte Weise mit der Mutterzellwand in Berührung, der andere Rand bleibt jedoch weit von der Mutterzellwand entfernt (Fig. 11). Unter diesen ungleichen Zuständen der Zellplattränder setzt die Bildung der festen Scheidewand

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Fig. 3–15. Serienaufnahme der Mitosenvorgänge von ein und derselben Zelle. In Fig. 4–14 ist der die mitotische Figur nicht enthaltende Teil des Zellraums ausgelassen. **3.** Photographiert 10.29 am 20. Mai 1940, neun Tage nach dem Aussäen[1]. Frühe Prophase. Man beachte die Lage des Kernes und die Größenverhältnisse des Kern- und Zellraums. **4.** 11.24. Mittlere Prophase. Anhäufung einiger Chloroplasten um die Kernwandung. **5.** 11.35. Späte Prophase. Im elliptischen Kernraum häuft sich das Atraktoplasma an beiden Polgegenden an, die Chromosomen jedoch in der Mitte. Die Kernoberfläche ist von dem sich bewegenden Zytoplasma verhüllt. **6.** 11.40. Metaphase. Man beachte die kontinuierliche Gestaltveränderung des Prophasekernes zur Metaphasespindel. **7.** 11.55. Frühe Anaphase. Ein Spindelpol und seine Polplasmastränge deutlich sichtbar. **8.** 12.05. Späte Anaphase. **9.** 12.10. Frühe Telophase. Man beachte die kontinuierliche Gestaltveränderung des Atraktosoms zum Phragmoplasten, in welchem die Bildung der Wandanlage schon beginnt. **10.** 12.20. Mittlere Telophase. **11.** 12.33. Späte Telophase. Das laterale Wachsen des Phragmoplast in der Vakuole. **12.** 12.40. Weiteres Heranwachsen des Phragmoplasten. **13.** 12.54. Die innerhalb der von den Tochterkernen isolierten Phragmoplastzone gebildete junge Wandanlage und die außerhalb derselben gebildete Scheidewand. **14.** 13.26. Die Zytoplasmatisierung der Phragmoplastzone

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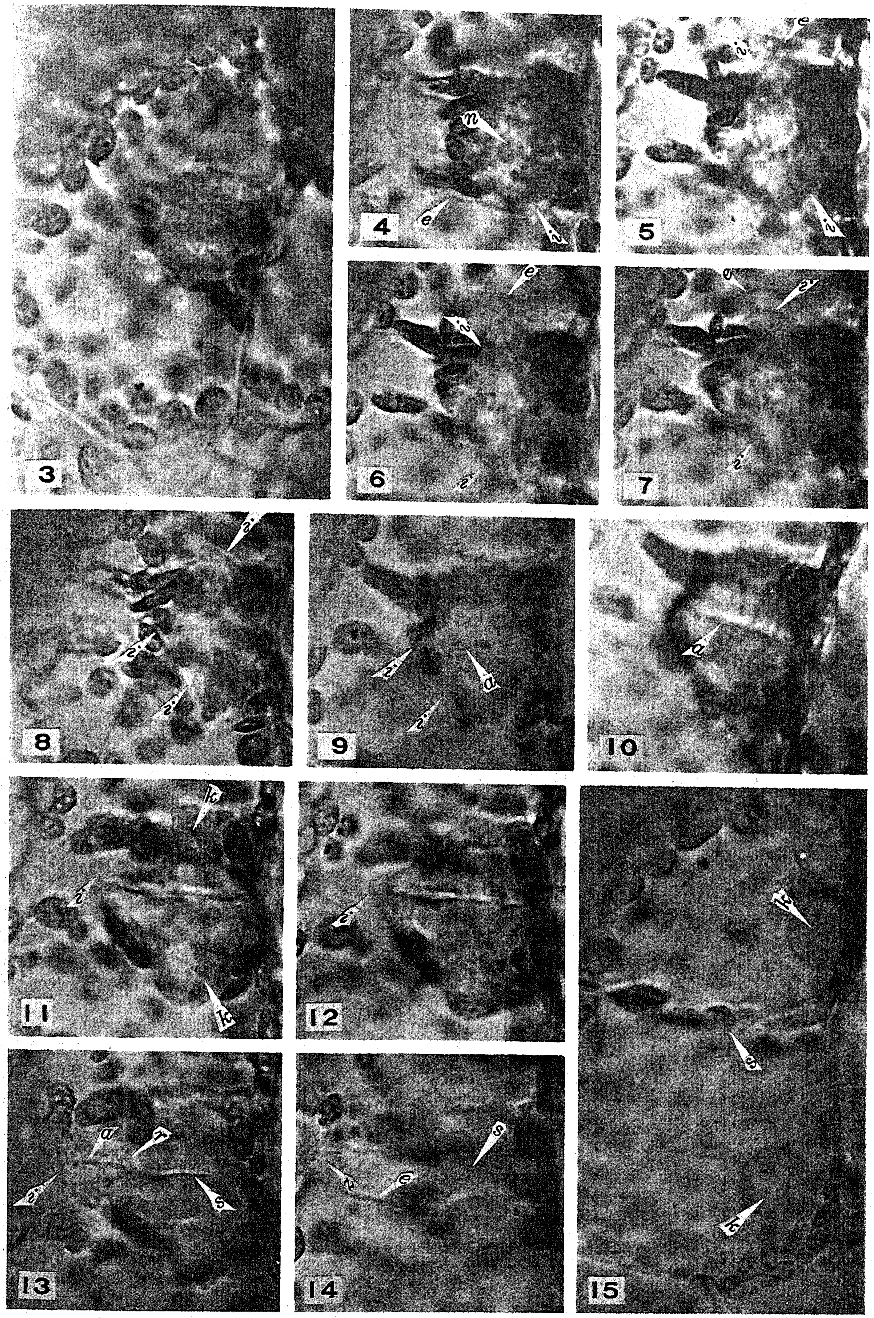
1) 10.29 bedeutet 10 Uhr 29 Minuten; die Worte Uhr und Minute sind (wie auch bei den folgenden Zeitangaben) weggelassen.



und das Übrigbleiben der dicken Plasmastränge. 15. 21. Mai, ein Tag nach der Mitose: *n* Nukleolus, *e* Zytoplasmastränge oder solche Anhäufung, *i* Grenzoberfläche des Atraktosoms und des Phragmoplasten, *a* Scheidewandanlage, *k* Tochterkern, *r* innere Grenzoberfläche der Phragmoplastzone, *s* gebildete Scheidewand, *z* Anhäufung der zytoplasmatisierten Phragmoplastsubstanz.

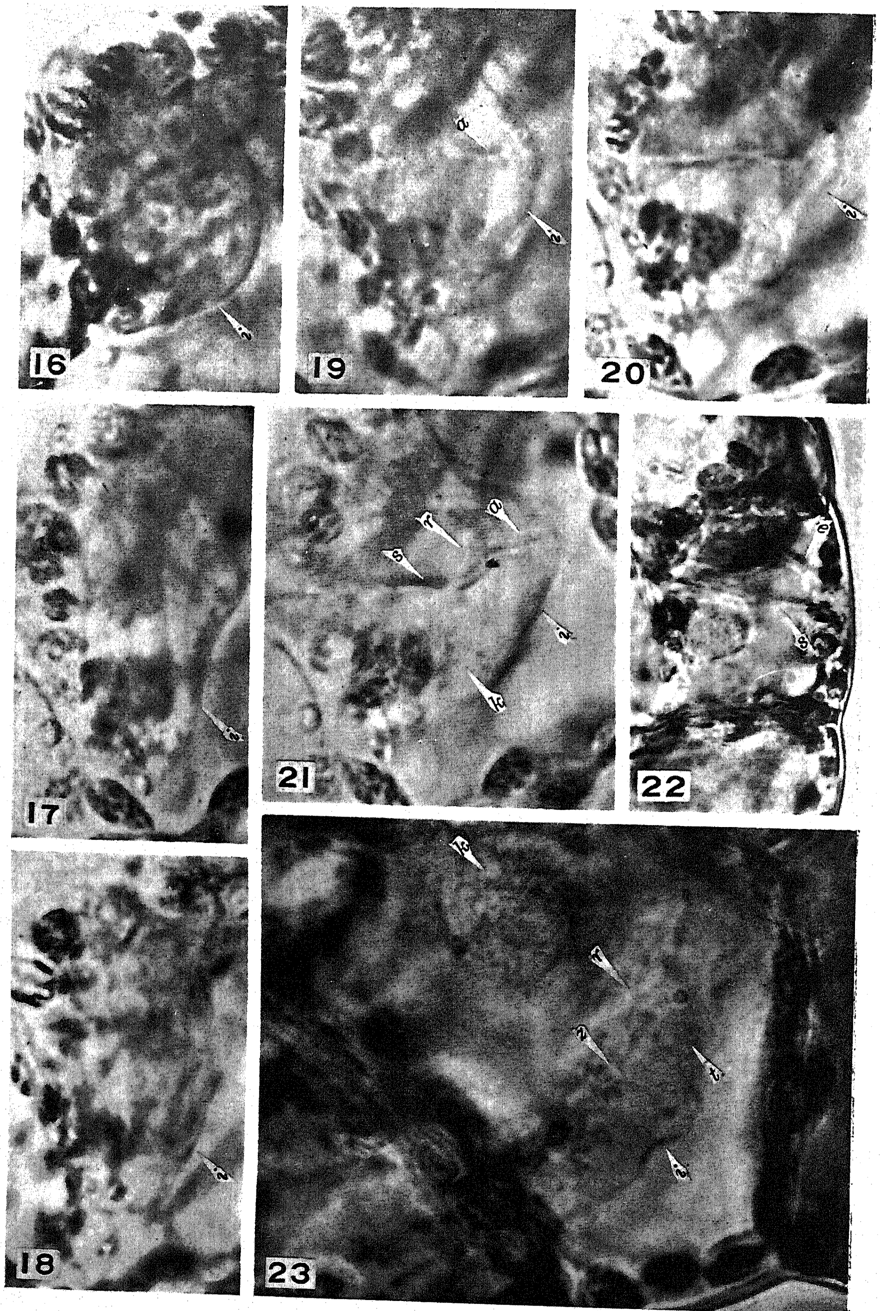


Fig. 16–22. Serienaufnahme der Mitosevorgänge von ein und derselben Zelle. Durch starke Vergrößerung sind die Wandungen der achromatischen Figuren anschaulich gemacht. In Fig. 16–21 ist der die mitotische Figur nicht enthaltende Teil des Zellraums ausgelassen. 16. Photographiert 15.45 am 6. Juni 1939, 16 Tage nach dem Aussäen. Späte Prophase. 17. 15.58. Frühe Anaphase. 18. 16.18. Mittlere

immer von einem Zellplattrand unter der Mitwirkung des Plasmawandbelags der Mutterzelle ein und wächst zum anderen hin.

Der Teil des Phragmoplasten, der im Zentralsaftraum liegt, dehnt sich nach der gegenüberliegenden Mutterzellwand aus (Fig. 12). Beim Wachsen des Phragmoplastteils spielt das Zytoplasma eine Rolle, wie bei der Bestimmung der Stelle und Achse der Spindelfigur. Zuerst treten einige Zytoplasmastränge um die Äquatorialzone des Phragmoplasten und an dem gegenüberliegenden Mutterzellwandbelag auf. Durch Strömung dieser Plasmastränge wird das Wachstum der Phragmoplastzone durch den Zellsaft erleichtert und zu einer bestimmten Lage gebracht (Fig. 13). Mit dem Wachstum der Phragmoplastzone dehnt sich auch die Wandanlage aus, wobei die feste Scheidewand durch die Mitwirkung des Zytoplasmawandbelags der Mutterzelle zentripetal und die neue Wandanlage fortsetzlich in der immer wachsenden Phragmoplastzone zentrifugal gebildet werden. Zwischen dieser jungen Wandanlage und der gebildeten festen Scheidewand bestehen daher verschiedene aber kontinuierliche Entwicklungsgrade der Scheidewand.

Dem zentrifugalen Fortschritt der Scheidewandbildung entsprechend verschwindet der Phragmoplastteil auch zentrifugal, wobei sich der Rest der Phragmoplastsubstanz zur Zytoplasmanatur verändert und schließlich mit dem Zytoplasma der Mutterzelle zusammenströmt. Die Zytoplasmatisierung der Phragmoplastsubstanz setzt parallel mit dem Beginn der Chromonematisierung der Tochterchromosomen von der Umgebung der Tochterkerne aus ein. Daher enthält eine späte telophasische Zelle einerseits eine sehr junge Wandanlage in dem ausgedehnten Phragmoplastteil, andererseits dagegen einen schon bestehenden Scheidewandteil, welcher mit der zytoplasmatisierten Phragmoplastsubstanz bedeckt und von den Tochterkernen durch Vakuolen getrennt wird (Fig. 13, 21). Die Zytoplasmaschicht, welche die äußere Oberfläche der Phragmoplastzone verhüllt und gestützt hat und dem Wachstum der Zellplatte in der Zentralvakuolen geholfen hat, bleibt oft in Form dicker Zyto-

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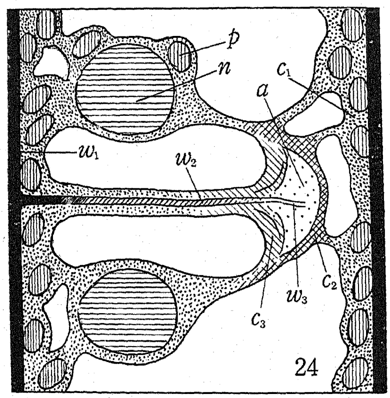
Anaphase. **19.** 16.30. Frühe Telophase. Die Wandanlage tritt im Phragmoplasten auf. Man beachte, das gleiche Aussehen der Wandung des Prophasekernes, des Atraktosoms und des Phragmoplasten, die sich kontinuierlich verändert. **20.** 16.40. Mittlere Telophase. **21.** 16.59. Späte Telophase. Das Heranwachsen der Phragmoplastzone in der Vakuole. **22.** 7. Juni, ein Tag nach der Mitose. **Fig. 23.** Polansicht einer späten Telophase. Zwischen die Tochterkerne und die Phragmoplastzone treten Vakuolen und die neue Scheidewand. *i* Grenzoberfläche des Atraktosoms und des Phragmoplasten, *a* Scheidewandanlage, *r* innere Grenzoberfläche des Phragmoplastzone, *s* gebildete Scheidewand, *k* Tochterkern, *z* Zone der zytoplasmatisierten Phragmoplastsubstanz, *t* Phragmoplastzone, *e* außerhalb der Phragmoplastzone gebildete Zytoplasmastränge.

plasmastränge noch lange Zeit übrig, nachdem die Zytokinese schon vollendet ist (Fig. 14, 22).

Im Vergleich der Scheidewandbildung bei den *Osmunda*-Prothalliumzellen mit derselben bei *Tradescantia*-Haarzellen scheinen die Zytokinesevorgänge der ersteren auffallend verwickelt zu sein. Jedoch bedarf die Scheidewandbildung bei den Haarzellen auch wesentlich derselben Vorgänge wie bei *Osmunda*-Zellen, nur was den Zellraum betrifft, gehen die Zytokinesevorgänge bei den Haarzellen fast gleichzeitig und auch allseitig vor sich. Daher ist es bei den lebenden Haarzellen unmöglich, die Zytokinesevorgänge analytisch zu verfolgen, ohne dabei die Zellen experimentell zu behandeln (vgl. WADA 1939). In *Osmunda*-Zellen gehen die Zytokinesevorgänge im normalen Zustande Schritt für Schritt und von einer Seite nach der anderen vor sich.

Da die sich bewegenden Zytoplasmateile um den Phragmoplasten oft eine scharfe photographische Aufnahme seiner äußeren Form verhindern, füge ich hier eine schematische Darstellung bei. Die Seitenansicht in Fig. 24 entspricht der Fig. 21 in Mikrophotographien und die Polansicht in Fig. 25 der Fig. 23. Durch Ver-



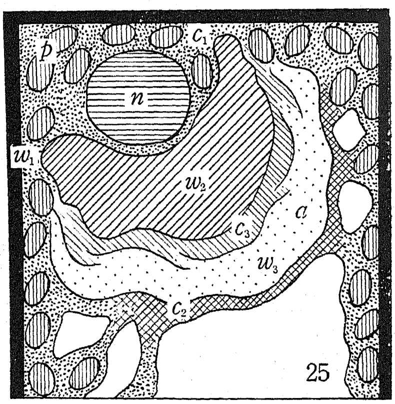


Fig. 24–25. Schematische Darstellung der Teilungsvorgänge in der späten Telophase. 24. Seitenansicht. 25. Polansicht: *a* Phragmoplast, *n* Tochterkern, *p* Chloroplast, w_1 Mutterzellwand, w_2 Scheidewand, w_3 Scheidewandanlage im Phragmoplasten, c_1 Zytoplasmawandbelag der Mutterzelle, c_2 Zytoplasmaschicht außerhalb der Phragmoplastzone, c_3 aus der zytoplasmatisierten Phragmoplastsubstanz entstandene Zytoplasmaschicht.

gleich der beiden Ansichten kann man die Zonenentwicklung der sich an der Scheidewandbildung beteiligenden Elemente richtig verstehen. Der Phragmoplast in Fig. 25 liegt schon von den Tochterkernen entfernt und bildet eine halbkreisförmige Zone. In der Seitenansicht verbindet diese aber die Tochterkerne mit den Plasmaschichten. Die Phragmoplastzone dehnt sich langsam zentrifugal zur Mutter-

zellwand aus; in derselben Weise dehnt sich auch die Wandanlage aus, die in der Phragmoplastzone immer weiter gebildet wird. Die äußere Oberfläche dieser Zone ist mit einer Zytoplasmaschicht bedeckt, welche nach der Zytokinese als Zytoplasmastränge übrigzubleiben pflegt. In der Innenseite der Phragmoplastzone verbreitet sich die gebildete Scheidewand durch den Zellsaftraum und die zytoplasmatisierte Phragmoplastsubstanz bedeckt die Innengrenzoberfläche der halbkreisförmigen Phragmoplastzone.

Schlußbetrachtung

Die intranukleäre Entstehung der Grundsubstanz der Spindelfigur, d.h. die Entstehung des Atraktoplasmas, die Gestalt des Atraktosoms, die morphologische Beziehung zwischen dem Atraktosom und dem Phragmoplasten und die Vorgänge der Scheidewandbildung—alle diese Verhältnisse, die ich (1935, 1939 u. a.) bereits bei der Mitose von *Tradescantia*-Haarzellen festgestellt habe, sind hier bei der Mitose von *Osmunda*-Prothalliumzellen durch Lebendbeobachtungen bestätigt worden. Besonders sind bei den *Osmunda*-Zellen sowohl die Gestaltveränderung des Prophasekernes zur Metaphasespindel als auch die der letzteren zum Phragmoplasten im lebenden Zustande anschaulicher als bei *Tradescantia*-Haarzellen, da die Teilungsfigur infolge des deutlichen Wachsens der Prothalliumzellen ihre äußere Form oft in der Vakuole unverhüllt darstellt. Jedoch bei dem Vegetationspunkte und bei ihm nahe liegenden kleinzelligen Geweben der *Osmunda*-Prothallien, wo die Mitosen häufig auftreten, sind die Volumenverhältnisse des Kern- und Zellraums ähnlich denjenigen bei den *Tradescantia*-Haarzellen. Das Atraktosom nimmt nämlich dabei fast die ganze Breite der Zelle ein und zeigt keinen freien Teil an seiner Grenzoberfläche; daher ist es sehr schwer, die äußere Form des Atraktosoms und seine weiteren Veränderungen zu beobachten.

Was die Bildung der Scheidewand bei der Mitose höherer Pflanzen anbetrifft, wurden Erklärungen bereits von vielen Forschern zu geben versucht (TREUB 1878, STRASBURGER 1880, TIMBERLAKE 1900, u.a.). Die Entwicklung der Scheidewand wurde aber von ihnen fast ausschließlich an fixierten Präparaten behandelt. Nur TREUB bemerkte schon damals bei lebenden Zellen einiger Pflanzen zwei Arten der Zellteilung; nämlich diejenige mit zentral gelegenen Kernen und Phragmoplasten und die mit exzentrisch gelegenen. Im letzteren Fall nahm er bereits wahr, daß die Zellplatte von dem Rande, der zuerst mit der Mutterzellwand in Berührung kam,

zu dem anderen hinüberwächst.[1] Neuerdings werden diese Probleme ebenfalls mittels Lebendbeobachtung erörtert und zwar wird die Mitose der *Tradescantia*-Haarzellen als wichtigstes Material zu ihrer Lösung benutzt (BĚLAŘ 1929, MARTENS 1927, BECKER 1938, YASUI 1939, SINNOTT und BLOCH 1940). YASUI untersuchte kontinuierlich die gesamten Vorgänge der Scheidewandbildung und erläuterte die Beziehungen zwischen der Zellplatte und der MLI (middle lamella initial). Die Zytokinesevorgänge wurden von ihr in zwei Phasen eingeteilt; nämlich in die Zweiteilung des Zytosoms während der ersten Phase durch das laterale Wachsen des Phragmoplasten und in die Zweiteilung des Phragmoplasten während der zweiten Phase durch dasjenige der MLI. Diese Vorstellung halte ich im Allgemeinen nicht für zutreffend, da in den stark vakuolisierten großen Zellen der *Osmunda*-Prothallium das Wachstum des Phragmoplasten, das der Wandanlage und das der festen Scheidewand nach einander vor sich geht und die Zweiteilung des Zytosoms oder des Phragmoplasten nicht von Bedeutung zu sein scheint.

Die Ansicht von SINNOTT und BLOCH über das Phragmosom bei der Mitose der *Osmunda*-Zellen trifft hier nicht zu, da die Differenzierung der Zytoplasmastränge, welche für das Phragmosom gehalten werden, bei den *Osmunda*-Zellen nicht zustandekommt. Ich bemerkte auch die lebhafte Tätigkeit der Zytoplasmastränge um den Prophasekern; sie bewegen sich jedoch nicht in einer bestimmten Fläche sondern fast in allen Richtungen. Dadurch beteiligen sie sich teils an der Bestimmung der Lage der Teilungsfigur gegen die Mutterzellwand, teils derjenigen der Spindelachse, aber nicht an das Zytoplasmaaggregat um den Phragmoplasten. Erst in der späten Anaphase fängt die Tätigkeit der Zytoplasmastränge sich an den Zytokinesevorgängen zu beteiligen an und erleichtert das Heranwachsen der Wandanlage durch den Zellsaft. Jedenfalls tritt die Scheidewand rechtwinklig in der Richtung zur Spindelachse auf, wobei es keine zytoplasmatische Vorbereitung vor dem Auftreten des Phragmoplasten gibt (Fig. 16–18). Durch Freihandschnitte ist es fast unmöglich, die Lage des Teilungskernes bei ein und derselben Zelle *in vivo* zu verfolgen; dasselbe gilt auch für fixierte Präparate. Bei ihren Materialien bemerkten SINNOTT und BLOCH auch die seitliche Lage der Prophasekerne und die, die Spindelpole mit der Mutterzellwand verbindenden Zytoplasmastränge. Die Frage über die Lagebestimmung der Scheidewand, welche sich auf die Richtung der Spindelachse bezieht, oder auf das Aggregat der Zytoplasma-

1) Zitiert aus der Arbeit TIMBERLAKES (1900).

stränge um den Prophasekern, müßte in Zukunft erst noch durch experimentelle Behandlung gelöst werden.

Die Bedeutung des lateralen Wachstums des Phragmoplasten besteht darin, daß der Phragmoplast durch das Wachsen die im Phragmoplasten fortsetzlich gebildete Wandanlage mit der Mutterzellwand in Berührung treten läßt. Wie ich (1939) bereits bei *Tradescantia*-Haarzellen experimentell festgestellt habe, setzt das Festwerden der Scheidewandanlage immer von derjenigen Seite der Mutterzellwand ein, wo die Wandanlage zuerst die Mutterzellwand erreichte, und schreitet dann weiter nach der anderen Seite fort. Im allgemeinen betragen meristematische Zellkerne, wie die der *Tradescantia*-Haarzellen oder die des kleinzelligen Teils der Prothalliumgewebe, mehr als zwei Drittel der Zellbreite und liegen in der Mitte der Zelle. Daher kommt der lateral wachsende Phragmoplast an seiner Peripherie allseitig und zwar gleichzeitig mit der Mutterzellwand in Berührung. Auch erreicht die Wandanlage auf die gleiche Weise die Mutterzellwand und formiert die Zellplatte; unter diesen Umständen erweist es sich als sehr schwer, das eigene Verhalten des Phragmoplasten und das der Wandanlage analytisch festzustellen.

In *Osmunda*-Zellen wurde die Kernteilung der Pollenmutterzellen bereits von vielen Zytologen untersucht; von ihnen beschäftigte sich SMITH (1900) mit der achromatischen Figur. Seiner Beschreibung wird entnommen, daß die achromatische Spindel gänzlich aus zytoplasmatischem Material (Kinoplasma) besteht, welches in der Metaphase durch das Verschwinden der Kernmembran mit der Kernflüssigkeit mischbar ist. Von STRASBURGERs Zeit an werden das Verschwinden der Kernwandung und die Mischbarkeit der Kernflüssigkeit mit dem Zytoplasma beim Auftreten der Metaphasespindel im allgemeinen einwandfrei anerkannt. Die Lebendbeobachtung der Mitose bei den *Tradescantia*-Haarzellen trug früher hauptsächlich zur Feststellung der Chromosomenbewegung bei, jedoch nicht zur Feststellung der Gestalt der Spindelfigur und ihrer Veränderungen. Jedenfalls bin ich jetzt zu dem Schluß gekommen, daß die Grenzoberfläche des Atraktosoms immer durch Fixierung, d.h. durch Entwässerung, gestört und unklar wird. Aus den Serien der Mikrophotographien in Fig. 4–9 und 16–19 wird ersichtlich, daß der Zustand der Wandung des Prophasekernes, des Atraktosoms und des Phragmoplasten nicht wesentlich von einander zu unterscheiden ist, daß der Inhalt dieser achromatischen Figuren zwar flüssig, aber sowohl mit dem Zytoplasma als auch mit dem Zellsaft nicht mischbar ist, und daß die Entwicklung der achromatischen Figuren immer durch kontinuierliche Veränderungen ihres Inhalts und ihrer Gestalt

vor sich geht. Gegenwärtig handelt es sich um die Lebendbeobachtung der meiotischen Kernteilung, da eine sichere Methode, durch welche man die in der Reduktionsteilung begriffenen Zellkerne, z.B. solche der Pollenmutterzellen, im lebenden Zustande beibehalten und zytologisch untersuchen könnte, leider noch nicht gefunden ist. Jedoch habe ich mich überzeugt, daß die oben erwähnten Ergebnisse bei somatischen Mitosen wesentlich mit dem Verhalten der achromatischen Figur bei der Reduktionsteilung der Geschlechtzellkerne übereinstimmen.

Nachdem FUJII (1931) das Atraktoplasma als die Grundsubstanz der Spindelfigur vorgeschlagen hat, wurde seine Ansicht durch Lebendbeobachtung bereits bei der Mitose von *Tradescantia*-Haarzellen bestätigt (WADA 1935 u.a., YASUI 1939). Aus der oben beschriebenen Arbeit ergibt sich jetzt, daß sich die Mitose bei den *Osmunda*-Prothalliumzellen auch für die Lebendbeobachtung des Atraktoplasmas als geeignetes Material erweist. Die Ergebnisse der Lebendbeobachtung der Mitose bei diesem Material haben uns die Bestätigung für die früheren Feststellungen geliefert, welche ich hinsichtlich des Verhaltens des Atraktosoms und des Phragmoplasten bei der Mitose der *Tradescantia*-Haarzellen *in vivo* durch Anstichversuche, durch das Dampfgemisch Ammonia-Chloroform und durch andere Behandlungen experimentell gemacht habe. Weiter bedarf es jetzt einer Richtigstellung der hauptsächlich auf Beobachtungen an fixierten Präparaten beruhenden im allgemeinen in botanischen Lehrbüchern aufgenommenen früheren Ansicht über das Verhalten der achromatischen Figuren.

Was das Verhalten der achromatischen Figuren betrifft, würde sich also die Mitose *in vivo* in folgender Weise abspielen: In der späten Prophase sammelt sich das aus der Karyolymphe entstandene Atraktoplasma innerhalb des Kernraums an seinen entgegengesetzten Polen, und die Chromosomen ordnen sich dabei von der peripherischen Lage zur Äquatorialgegend des Kernraums an. Während dieser inneren Verlegung verändert der Prophasekern kontinuierlich seine äußere Form von einer kugeligen zu einer elliptischen und weiter zu einer spindelförmigen, und er entwickelt sich daher ohne Verschwinden seiner Wandung zu dem Atraktosom. Die Differenzierung der Spindelfasern geht ausschließlich innerhalb der Begrenzung des Atraktosoms vor sich. Abgesehen von der Chromosomenbewegung verändert sich das Atraktosom in der späten Anaphase kontinuierlich durch Anschwellung an seiner Äquatorialgegend zum tonnenförmigen Phragmoplasten. Dann entwickelt sich die Scheidewandanlage im Phragmoplasten zentrifugal, aber die feste Scheidewand durch Mitwirkung des Zytoplasmawandbelags der Mutterzelle zentripetal. Aus

diesen Vorgängen ergibt sich, daß sich sowohl das Atraktosom als auch der Phragmoplast *in vivo* vom Zytoplasma sich abgrenzend als ein abgeschlossener Körper verhalten. Erst nach der Bildung der Zellplatte kommt der Abbau des Phragmoplasten durch Zytoplasmatisierung der sich an der Bildung der Scheidewandanlage nicht beteiligten Phragmoplastsubstanz zustande.

Zusammenfassung

Das Verhalten der achromatischen Figur wurde bei der somatischen Mitose der jungen Prothalliumzellen von *Osmunda japonica* THUNB. *in vivo* untersucht.

Aus den Lebendbeobachtungen ergibt sich, daß weder das Verschwinden der Kernwandung am Ende der Prophase noch das Vermischungsvermögen der Kernflüssigkeit mit dem Zytoplasma beim Auftreten der Metaphasespindel stattfinden, daß sich die Wandung des Prophasekernes dabei kontinuierlich zu derjenigen des Atraktosoms und weiter zu derjenigen des Phragmoplasten verändert, und daß sich das als Grundsubstanz der achromatischen Figur aus der Karyolymphe entstandene Atraktoplasma hinsichtlich seiner Gestalt und seiner Funktion vom Zytoplasma sich abgrenzend als ein abgeschlossener Körper verhält.

Die Scheidewandanlage entwickelt sich im Phragmoplasten zentrifugal, die feste Scheidewand jedoch durch Mitwirkung des Zytoplasmawandbelags der Mutterzelle zentripetal, wobei die Scheidewandbildung bei stark vakuolisierten großen Prothalliumzellen von der einen Seite der Mutterzellwand zu einer anderen fortschreitet. Nach der Bildung der Zellplatte degeneriert der Phragmoplast durch Zytoplasmatisierung der sich an der Bildung der Scheidewandanlage nicht beteiligten Phragmoplastsubstanz.

Zum Schluß möchte ich hinzufügen, daß ich der Japanischen Gesellschaft zur Förderung der Zytologie für die finanzielle Unterstützung dieser Arbeit zu großem Dank verpflichtet bin.

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Chromosome Studies on *Trillium kamtschaticum* Pall. XIII. The structure and behavior of the kinetochore[1)]

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Introduction

It is now generally accepted that the kinetochore plays an important rôle in mitosis. The prime function of the kinetochore lies in that it is directly responsible for the movement of the chromosome from the equator to the pole, though exceptions to this rule are known in a few special cases, viz., *Sciara* (Metz '33) and *Micromalthus* (Scott '36). In this sense the kinetochore is the "motor chromosomalis" (Lorbeer '34); it is, so to speak, the eye of the chromosome. The justisfication of this idea is further evidenced by the behavior of a chromosome when it happens to lose its kinetochore (resulting in an akinetic fragment) or to gain an extra kinetochore (forming a dikinetic chromosome). For the accomplishment of the normal mitosis, however, there is necessary a certain concordant timing relationship between the kinetochore cycle and the cycle of the rest of the chromonema. Upsets in this relation may cause several abnormalities in the division (cf. Nos. 5 and 8 of this series). Beside these functional features, the kinetochore is known to be morphologically characterized by its definite location on chromosomes, by its differential staining capacity from the rest of the chromosome (cf. Schrader '36, '39b), and by the attachment of the spindle with it. Furthermore structurally the kinetochore is known to be more resistant to X-rays than the rest of the chromonemata, as many X-ray experiments suggested. All these facts point to the conclusion that the kinetochore is a definite and permanent organ of the chromosome and differs from the rest of the chromonema both functionally and structurally.

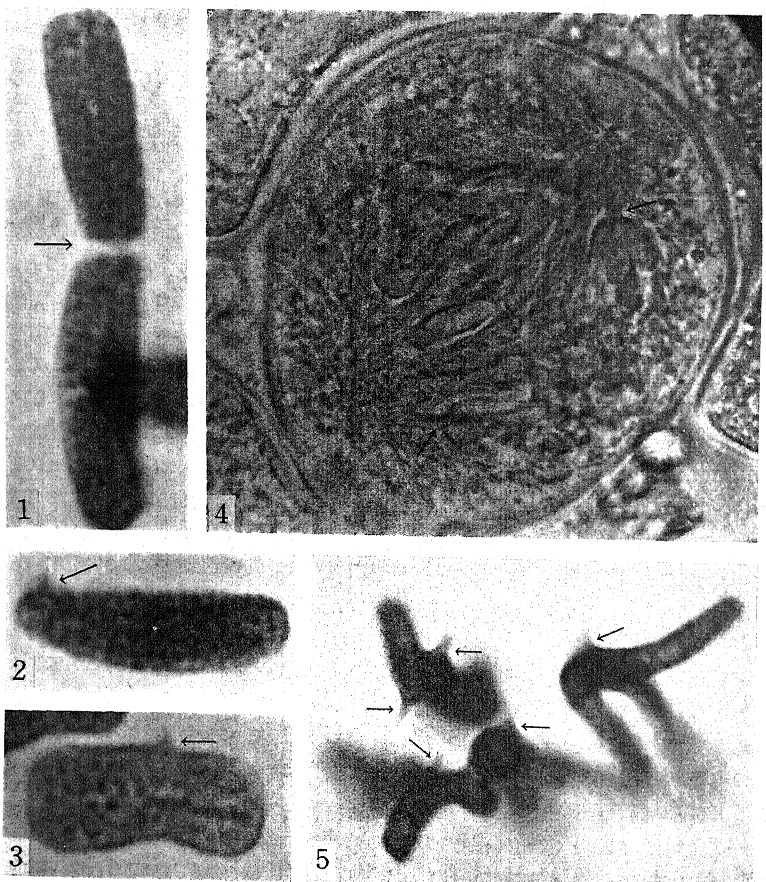
Although such great importance is to be ascribed to the kinetochore, our present knowledge on its detailed structure is very unsatisfactory. This is supposed to be partly due to technical difficulties owing to its minuteness in size in general and partly due to its variable appearances at different stages in its cyle and also under

1) Aided by a grant from the Japan Society for the Advancement of Cytology.

different treatments. Most of the observations hitherto made are necessarily very fragmentary. Especially the structure of the kinetochore in relation to the rest of the chromonema remains still as an open question, though some tentative interpretations have been made on this point by Schrader ('36, '39b), Nebel ('39), Darlington ('39) and others. The present study was made with a purpose to fill this gap.

Variable effects of fixation

The most serious difficulty in the study of the kinetochore seems to lie in the variable effects of fixation (cf. Schrader '39b, Iwata '40).



Figs. 1–5. Representing variable appearances of the kinetochore. 1–3, chromosomes A, C and E, respectively, at metaphase in pollen grain mitosis, treated with acetocarmine. 4, a PMC at first anaphase which was fixed in very dilute acetic acid. 5, bivalents at first metaphase in a PMC, taken from a permanent smear preparation (La Cour-gentian violet). Arrows indicate the kinetochore regions. 1–3, ×ca. 3560; 4, ×ca. 1320; 5, ×ca. 1800.

In usual methods of fixation, it generally appears as an achromatic or very faintly staining gap in the chromosome, through which one or two fine connecting threads are often observable (Fig. 1). Such a condition is usually met with in somatic chromosomes and is one very familiar to a great many cytologists under the name of the primary constriction of the chromosome. Sometimes from this region is made a protrusion which is conical in shape, orientates polewards and is usually stained faintly. Often the top of this cone is provided with a tiny granule which is usually more deeply stained. Such a conical protrusion is observed in both somatic and meiotic metaphase chromosomes (Figs. 2, 3 and 5). In preparations in which the spindle is well fixed, one can see the condition that a bundle of spindle fibers is tightly connected with the kinetochore region (as indicated by the arrows in Fig. 4).

Such diverse appearances of the kinetochore as mentioned above may be taken as representative descriptions of its fixation images with which one usually meets in current literature. The writer believes that in these cases one is concerned with what Bělař called a vital artefact, and that the real structure of the kinetochore must be of a nature to cover all these images and to warrant a generalisation. As will be described later, the writer's water-pretreatment method of fixation proved to be quite satisfactory to reveal the real kinetochore structure.

Confusion in terminology

Such diversity in appearance of the kinetochore and consequent lack of adequate knowledge of its structure has led to serious confusion in terminology of this cell organ. Names hitherto applied to the kinetochore as a whole or apparently to one of its components are numerous as summarized in the following*:

Constriction:
Primary —, kinetic —, attachment —, centric —, spindle fiber attachment —. (Einschnürung, kinetische —, Querkerbe, Querspalte, Transvasalspalte, chromatische Spalte, Trennstelle, Gelenk, Umbiegungsstelle).
Commissure.
Achromatic region; Achromite.[1]
Spindle fiber locus.[2]
Attachment:
Spindle —, fiber —, fiber—point, spindle fiber —, — region, spindle—region, —body. (Spindelfaseranheftung, Anheftungsstelle).
Insertion:
— region, spindle fiber —. (Insertionsstelle, Insertionspunkt, Insertionslücke).
Kinetic body.
Kinetochore.
Centromere; Attachment chromomere.

Polar granules; Spindle spherule[3]; Leitkörperchen; Granule proximale. Traction cone (Zugkegel).[4]

* Regarding authorities of these terms, cf. Lorbeer ('34) and Darlington ('37, p. 536), excepting, [1]) McClung (ex Schrader '35), [2]) Metz ('33), [3]) Schrader ('36, '39b). [4]) Fujii & Yasui ('35).

Most of the present geneticists use the name, spindle fiber attachment (abbreviated as *sfa*). Among cytologists there are two main schools, one adopting "centromere" which Darlington invented and the other adopting "kinetochore" which Sharp recommended. Recently, between Schrader ('39a) and Mather ('39) a discussion has arisen as to which of the two is better. As far as the writer's observations are concerned, the term "kinetochore" seems to merit more justification, because its morphological features prove to be different far from the concept that the "centromere" implies (v. infra).

Division of the kinetochore

In prophase stages of meiosis, the kinetochore appears as a 'naked', fine, less deeply staining thread connecting the chromonemata at both sides. This condition is especially clear when the meiotic chromosomes are 'precocious' as previously illustrated in Figs. 7–9 of paper, No. 5, of this series. Towards metaphase it develops as an organized body, but when this development is prevented somehow, the 'naked' condition remains even in later stages. Fig. 6 probably represents such a situation. In this case X-rays had effects, besides chromosome fragmentation and fusion, causing the retardation of the normal organisation of the kinetochore, thus resulting in its precocious division (into the daughter kinetochores) without going to the poles.

The following description on the structure and behavior of the kinetochore at meiotic metaphase is principally based on a study on permanent smear preparations (La Cour 2BE—gentian violet) to which the water-pretreatment after the writer's method (No. 11 of this series) was applied. The manner of kinetochore division is represented in Figs. 7–17 and diagrammatically in Fig. 16 a–f.

The conjugated kinetochores at early metaphase appear as a single body staining more deeply, not less deeply at least, than the rest of the chromonema (Figs. 7, 16a). The body measures ca. 1.5×3 μ. This size stands in marked contrast with the statement of Darlington ('37, p. 536) that "in the largest chromosomes it appears to be 0.2 μ in diameter with a chromo-acetic fixation, and larger with acetocarmine". Such a great discrepancy in the size of the kineto-

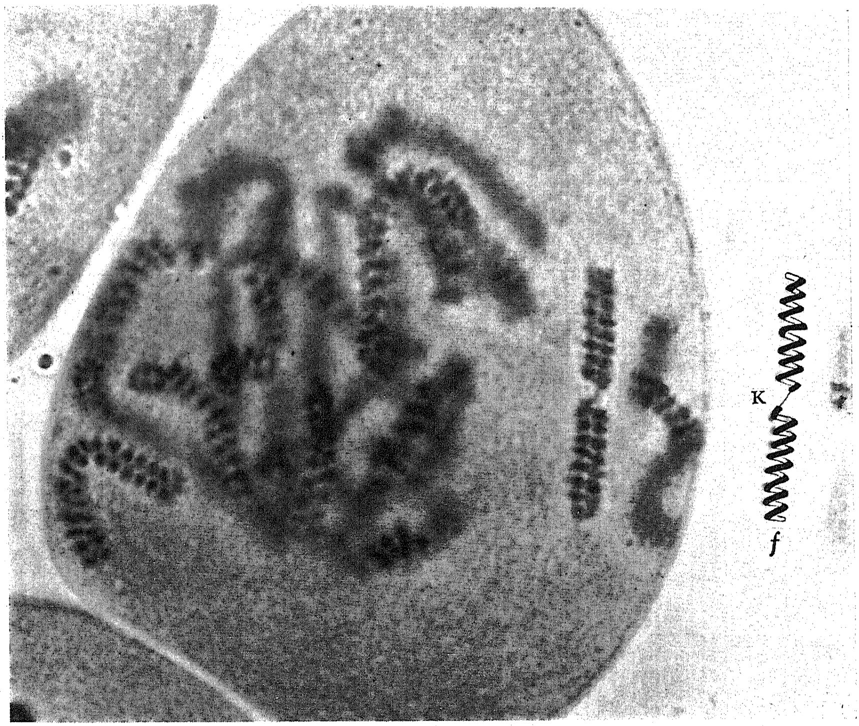
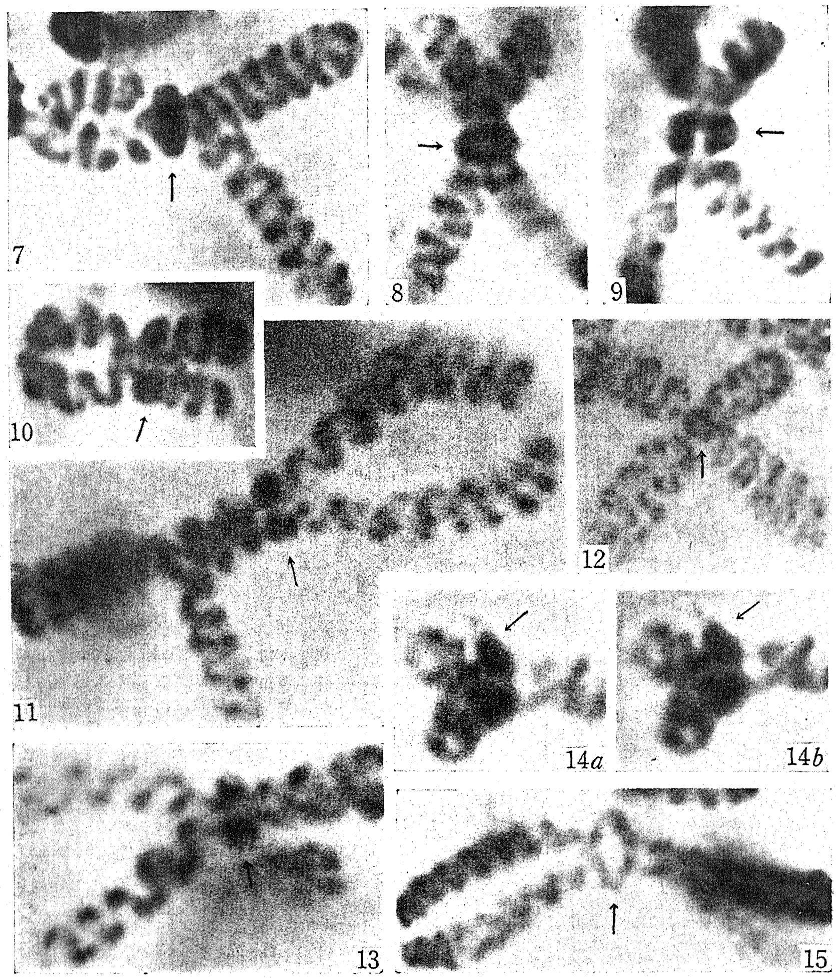


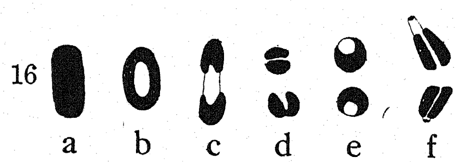
Fig. 6. An X-rayed PMC. Note the failure of the spindle mechanism and the precocious division of the chromosomes (into the chromatids). A kinetic chromatid fragment shown on the right side of this cell is represented by an attended explanatory sketch (f). K indicates the kinetochore. Acetocarmine, pretreated with water. ×ca. 1800.

chore is based on the fact that the present writer is concerned with the kinetochore as a whole, that is, as will be described later, with the matrix of the kinetochore, while Darlington is dealing with the so-called "polar granule". Be that as it may, it may be pointed out that the kinetochore of the present material is exceedingly great in size, as will be understood when one recalls that in other organisms there are chromosomes less than 1 μ in their whole length.

The body is spherical in shape in front view, as represented in Figs. 7 and 16a, but in side view it is found to be ring-shaped as in Figs. 8 and 16b. The mode of separation of the kinetochore is considered as essentially the same as that of the rest of the chromonema, with a difference in time of its operation, viz., in the former it takes place at metaphase, while in the latter it is at diplotene (v. infra). The paired kinetochores 'open out' into a pair of paired daughter kinetochores, each chromatid being thus provided with a kinetochore. Very often the divided kinetochores are found to be connected with fine threads, a condition previously represented in

Fig. 1 of the third paper of this series and here in Figs. 9, 10, 16c. A very characteristic feature of the kinetochore at this time of division is the development of a 'bubble' within it (Figs. 11–13,





Figs. 7–16. Representing the mode of division of the kinetochore at MI; Fig. 16, its diagrammatic representation. Photos, 7–14, are taken from PMCs pretreated with water; photo 15 from a preparation to which natural dessication was applied as the pretreatment. All the photos, excepting Fig. 12 (acetocarmine), were taken from permanent smears. Chromosome type: 7–9, **B**; 10, **E**; 11, **A**; 12, **E**; 13, **B**; 14 a and b (taken at two different foci), **E**; 15, **A**. The arrows indicate the kinetochores. All the photos, ×ca. 3030, excepting Fig. 12, ×ca. 2230.

16de). It orientates poleward, and appears to be 'pulled up' by the polar attraction, with the consequence that a portion of the kinetochore becomes considerably attenuated into a fine thread. The apex of this connecting thread is usually provided with a tiny spherule (Figs. 14ab, 16f), which has been called the "polar granule", the "spindle spherule", etc. by other investigators. The spherule seems however to be very unstable and to vary in size to a certain extent in different treatments. The writer inclines therefore to consider it dubious that such a spherule is of a permanent organisation.

At late anaphase the paired daughter kinetochores begin to separate from each other, as previously figured (Fig. 8, No. 3 of this series); they then enter interkinesis and at metaphase II take the poleward orientation of the second division.

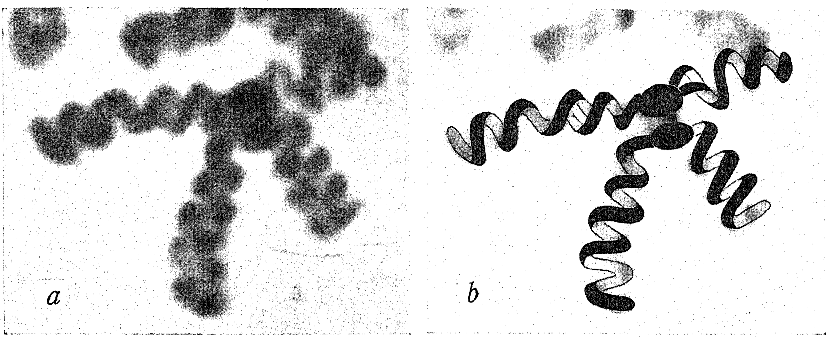


Fig. 17. Representing the internal structure of a whole bivalent E at early metaphase. Fig. b was drawn from the original photo a. From a permanent smear preparation pretreated with water. ×ca. 3460.

Internal structure of the kinetochore

Based on the above findings, one can understand the relation of the kinetochore to the chromonema in a complete bivalent at early and late metaphases to be such as diagrammatically represented in Fig. 18ab. Here one is concerned with the kinetochore as a whole and the next question to be raised is regarding its internal structure.

The writer infers from observations on the development of the kinetochore that the essential element persistent throughout the kinetochore cycle is the fine thread, chromonematic in structure but different in behavior (v. infra), which may be referred to as the *kinetonema*. At early stages of the division the kinetochore consists of the naked kinetonema; at metaphase the latter is provided with a matrical substance. That the above observations are con-

cerned with the behavior of the kinetochore matrix, not directly with that of the kinetonema, may be evidenced by the fact that in PMCs to which the natural dessication was applied before fixation, this matrix does not take the stain and the kinetonema can be clearly discerned (Fig. 15). This paired kinetonemata open out at metaphase in the same way just as the rest of the chromonemata does at diplotene. They open out both reductionally and equationally, and each of a paired daughter kinetonemata repels each other and comes to orientate poleward. Then within the kinetochore matrix a fluid droplet is supposed to be excluded and to spread poleward along the spindle, originating thus the so-called "Zugfasern." By this streaming-out of a droplet, a portion of the kinetonema will be considerably attenuated towards the pole. These situations are diagrammatically represented in Fig. 19. Such an idea as here mentioned of the origin of "Zugfasern" is on the same line as Bělař (29) previously advocated.

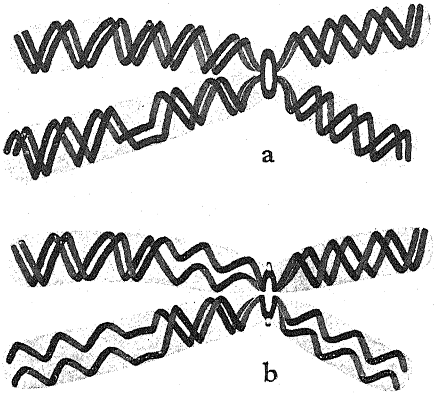


Fig. 18. Diagrammatically illustrating the kinetochore-chromonema relationship in whole bivalents at early metaphase (a) and at late metaphase (b). Note: the arms are independent of each other in the direction of chromonema spirals and the paired chromatids are coiled relationally at a but parallel at b (cf. No. 12 of this series).

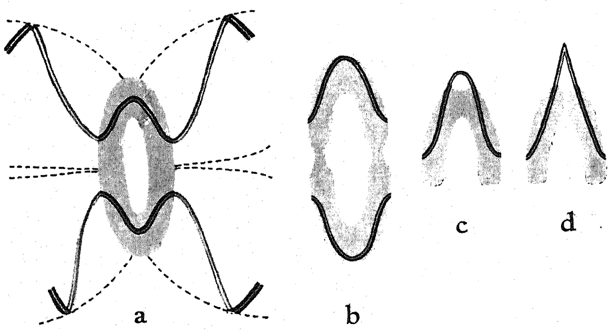


Fig. 19. Diagrammatically illustrating the internal structure of the kinetochore at MI and its behavior. a shows the connection of the kinetonema with the genonema, the matrix of the former being pale colored and that of the latter dotted-outlined. In order to avoid confusin, the opening-out of the paired kinetonemata is assumed to take place in the same plane as the genonemata, and further the minor spirals of the latter are omitted. b-d illustrate the division of the kinetochore matrix and the reaction of the kinetonema to polar attraction.

Since the kinetochore is the same in its structure and behavior in every chromosome within a complement, that is to say, homologous

in this sense, it is reasonable to distinguish the kinetonema from the rest of the chromonema. It seems to be fitting to refer to the latter as the *genonema* (Koltzoff '34).

The matrix surrounding the kinetonema may remain unstained in different treatments as in the matrix of the genonema. Nebel ('39) called the main body of the kinetochore the "central achromatic body", but as mentioned above, it is not always achromatic. When it is achromatic and the other part of the chromosome is deeply stained, the kinetochore region will appear as a constriction. The spindle spherule as described by Schrader ('36, '39b) is considered to be of a dubious nature, because one can not adequately conceive how this body is connected with the chromonema and further it seems probable that his method "overfixation" may have caused a considerable contraction of the kinetonema. Such tiny spherules, including polar granules of other authors, are believed to be of an artefactual nature and not of a permanent organisation.

Posteriority in behavior of the kinetochore

The essential characteristic of the kinetochore is in its reacting to the polar attraction; otherwise the kinetonema behaves just like the genonema, excepting that there is a certain retardation in the kinetochore cycle as contrasted with the cycle of the genonema. This inference is based on the following facts: (i) the paring of the homologous genonemata takes place at zygotene, while that of the kinetonemata at diaphase, (ii) the opening-out of the paired genonemata (into two-by-two chromatids) takes place at diaphase, while that of the paired kinetonemata (into two-by-two daughter ones) at metaphase, (iii) the separation of the paired chromatids occurs at late MI, while that of the paired kinetonemata at late AI, (iv) the matrix development begins at diaphase in the genonema, while it occurs at a later stage approaching metaphase in the kinetonema. This posteriority or serotinous nature of the kinetochore is of prime significance in accomplishing the normal division. It was pointed out in previous reports (Nos. 5 and 8) that upsets in this timing relationship lead to several abnormalities in the behavior of chromosomes.

Structurally the kinetonema is suggested to be essentially the same as the genonema. That it is multiple in nature, as suggested by Nebel ('35), Schrader ('36,'39b), Darlington ('39) and Iwata ('40), is no longer disputable. Essentially each chromonema is supposed to have its own kinetonema. For the condition in which two divided genonemata are nevertheless held together the posterior

nature of the kinetochore behavior is responsible. They are held together either by the still undivided kinetonema, or after its division, by the still undivided matrix of the kinetochore. These two conditions are actually responsible for the fact that two daughter chromatids in mitosis and two homologous chromosomes in meiosis are held together up to metaphase.

Summary

1) It is pointed out that the kinetochore is subjected to variable effects under different treatments and this has caused much serious confusion in terminology of this cell organ. The writer prefers the term "kinetochore" for this organ rather than the other current name "centromere", because it proved to be not of such a chromomeric nature as the latter name implies.

2) The kinetochore is a compound body consisting of the chromonematic thread (=the kinetonema) which is persistent throughout the division and of the matrix surrounding it which develops fully at metaphase.

3) The behavior of the kinetonema is just the same as that of the rest of the chromonema (=the genonema), viz., in pairing, in opening-out, in separation of the daughter halves and in the development of the matrix, excepting that the genonema is always precocious in behavior as contrasted with the kinetonema under usual conditions.

4) The origin of the so-called "Zugfasern" is inferred to be such as previously advocated by Bělař; they arise from the kinetochore toward the pole and not in the reverse direction.

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Chromosome Studies on *Trillium kamtschaticum* Pall.
XIV. Primary and secondary chiasmata[1]

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The results from an experimental study on the chiasma formation in *Trillium kamtschaticum* undertaken by the present writer and T. Haga were interpreted as favoring the two-plane theory of its origin (No. 10 of this series). The writer considers from this and other unpublished sources of evidence that the mode of chiasma development is as follows. The synaptic association of the homologous chromosomes at zygotene *usually* begins at their distal ends and proceeds towards the proximal ends. Thus the pairing process terminates at the kinetochores. Likewise the opening-out of the chromatids at diplotene *usually* originates at the distal ends and proceeds proximally. Thus the separation of the paired kinetochores takes place as the final event. With respect to these behaviors, the kinetochore is thus always later in time than the rest of the chromonema (cf. No. 13 of this series). In such *normal* case the plane of chromatid opening-out which was first determined at the distal ends by the repulsion between the pair of paired chromatids will continue throughout the paired arms and consequently no chiasma will be formed, the pair of paired chromatids being associated only at the kinetochore region and forming characteristic cross-shaped figures. On the other hand, when the meiotic division is accelerated by e.g. heat, certain discrepancies may occur in the mode of pairing and subsequent repulsion; repulsion may set in interstitially.

When repulsion begins at two different intercalary points and the planes of opening-out of the chromatids are different in these two sections, the result is the formation of one interstitial chiasma. Thus the frequency of chiasmata per arm pair is taken as a function of these beginning points of repulsion.

Supporting the above view of chiasma development, an interesting fact has been revealed this time from observations on PMCs which were taken from plants placed in an incubator of 20°C for about a week till meiotic metaphase began. In the present study La Cour 2BE-Gentian violet smears, pretreated with water according to the writer's method (No. 11 of this series), were used.

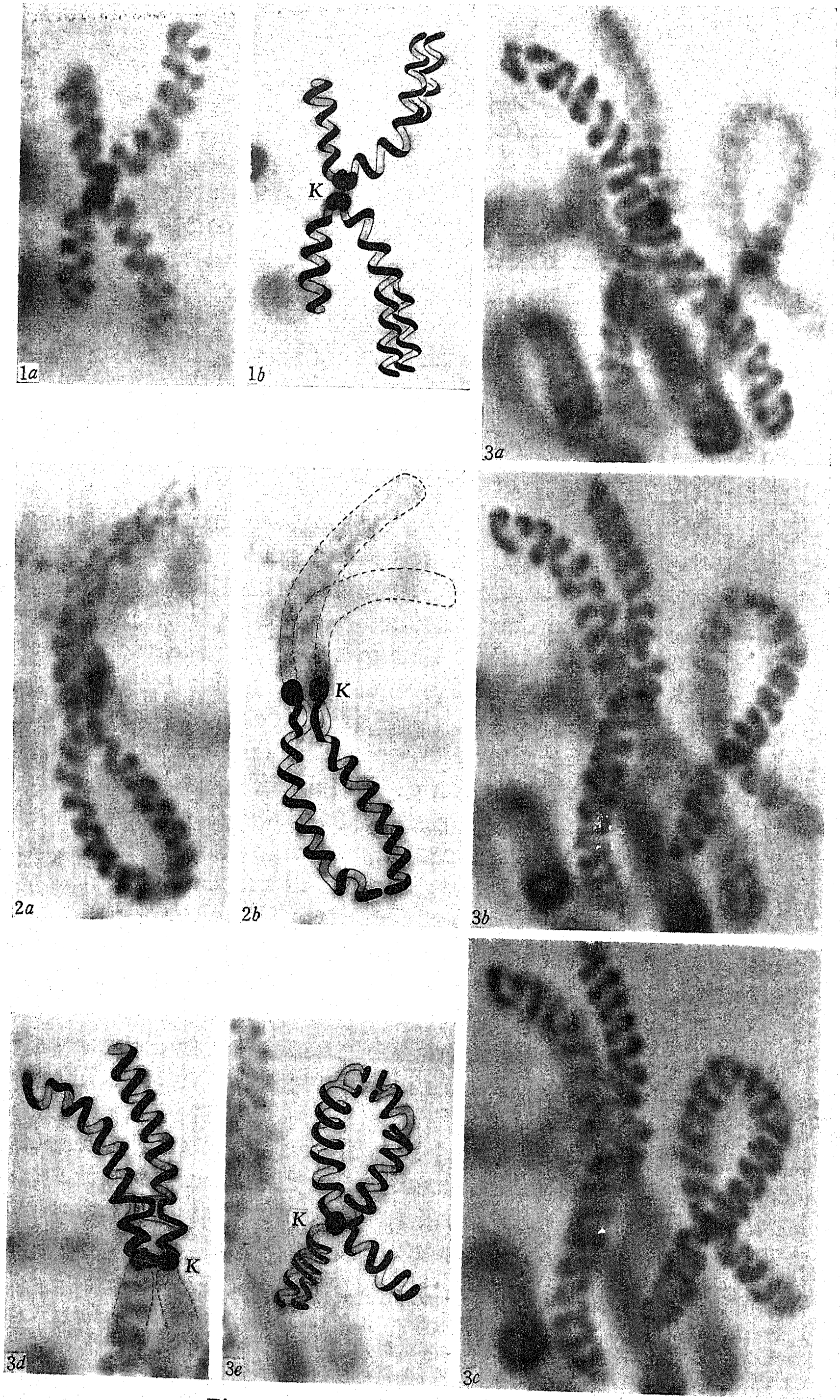
1) Aided by a grant from the Japan Society for the Advancement of Cytology.

In this material the association of the kinetochores is complete and the frequency of chiasmata is rather high, like the t_1 type described in a previous paper (No. 10 of this series). Very remarkable is the very frequent occurrence of small chiasma loops which are located either proximally neighboring the kinetochore or interstitially close to a larger loop. These situations are illustrated in Figs. 2–4. Likewise close to the distal ends of paired arms when associated together, a chiasma as shown in these figures is very frequently found. These small chiasma loops are discernible only when the chromonema structure of chromosomes is quite obvious as in these photos, and this is the reason why no particular description of such chiasmata has been hitherto made.[1] Now how these small chiasma loops were brought about is the subject matter to be considered here.

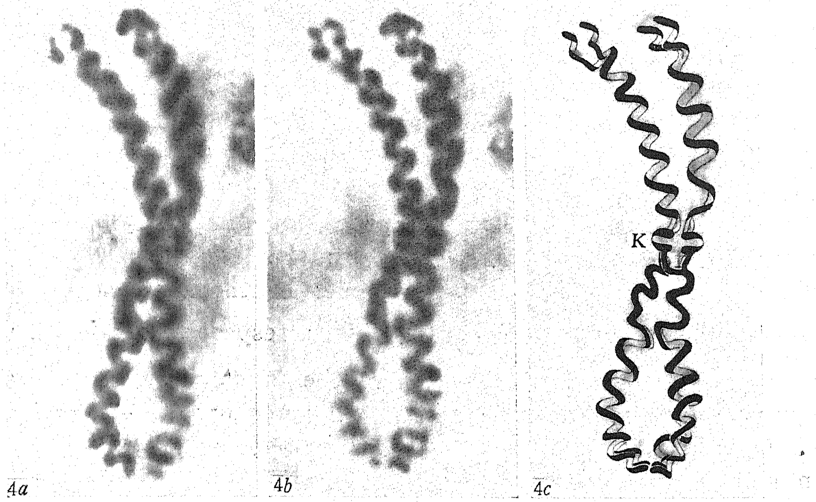
In the writer's view of chiasma development, these small loops must have been caused by different openings-out independent of one in neighboring larger loops, but to what should the peculiarity that these loops are always so small be attributed? The writer believes it reasonable to ascribe this peculiarity to the equilibrium between the strength of repulsion at the time of opening-out on the one hand and the surface tension and the viscous resistance set up by the matrix substance on the other. This implies that if the chromatid opening-out takes place after the matrix became viscous to such a degree that certain accumulation of the matrix substance will occur at these junctions—a condition supposed to be analogous to the case when two appressed fingers are dipped into mucilage and then separated slowly so that the aggregation of mucilage will occur at the root-junction of the fingers—, the portion of the chromatids lying within such unseparated matrix will constitute a system independent with respect to chromatid opening-out of the neighboring loop.

This relation of repulsion to matrix resistance is diagrammatically represented in Fig. 5. When repulsion occurs at two different interstitial points, A and B, within an arm-pair (Fig. a), two corresponding principal loops, A and B, will be formed at diplotene and accompanying these loops the accumulation of the matrix at the junctions will bring forth three small regions, proximal (x), interstitial (y) and distal (z), which are no longer under the effect of the

1) In the study on meiotic chromosome structure in *Trillium erectum*, Huskins and Smith ('35) state: "it is noteworthy that chiasmata very frequently occur immediately adjacent to and on both sides of the attachment." Most probably these chiasmata correspond with those in the present question. Moreover in some of their figures, e. g., Figs. 11 and 12, Pl. II, interstitial small loops are clearly shown, though they made no remark on this point.



Figs. 1-3. For explanation, see p. 383.



Figs. 1–4. Representing various configurations of bivalents; 1 showing no formation of chiasmata, 2-4 showing various types of formation of proximal, interstitial or terminal chiasmata. Chromosome type: 1, 3e, B; 2, 3d and 4, A. Figs. 3a, b and c and Figs. 4a and b were taken at different successive foci. K indicates the kinetochore. ×ca. 2420.

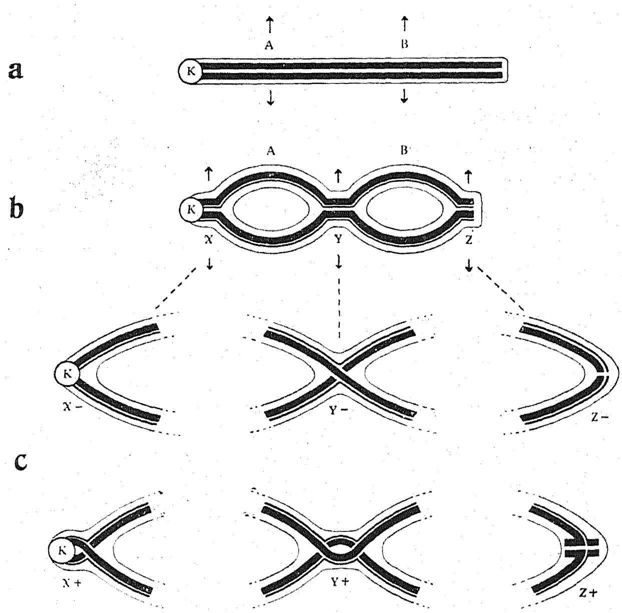


Fig. 5. Representing the development of proximal (x), interstitial (y) and distal (z) junctions (a and b) and resulting figures of opening-out of chromatids in these regions (c). In this diagram only one pair of arms is represented. k represents the kinetochore.

original repulsion forces (Fig. b). Within these junctions, the chromatids will then take their own ways of opening-out, as illustrated in Fig. c. Based on the two-plane theory of chiasma development, the formation or non-formation of chiasmata in these regions can be statistically treated. The numerical data obtained will be given in the following under separate headings, viz., (i) proximal, (ii) interstitial and (iii) distal junctions.

i) Proximal junction. The probability that the plane of opening-out differs in the two regions, x and A, thus resulting in the formation of a chiasma at the border of them, is calculated as $\left(\frac{1}{3}\times\frac{2}{3}\right)\times 3$, that is, $\frac{2}{3}$. Accordingly with respect to bivalents, those having chiasmata on both sides of the kinetochore (++), those having a chiasma on one side of the kinetochore (+−), and those having no such a chiasma (−−) are expected to occur respectively in a 4:4:1 ratio. Since it was quite difficult to analyze all the members of the complement, the writer selected at random favorable chromosomes of the **A, B, C** and **E** types in which both arms of a bivalent are analyzable, and careful records were made on them. Observations on a total of 114 bivalents showed the following results:

Chromosome type	++	+−	−−
A	14	19	4
B	20	12	6
C	8	4	5
E	8	9	5
Total	50	44	20

The results indicate that the observed ratio per 9 for the ++, +− and −− classes is 3.95 : 3.47 : 1.58, the deviation from the expected ratio being ±0.58. When individual arms are separately treated, the frequency of the two classes, + and −, is 94 and 64 respectively, i.e., a ratio of 2.78:1.22 per 3. Such a rather large discrepancy of the actual data from the expectation will be ascribed to a considerable excess of the −− class in the present records. Most probably this is due to greater ease in analysis and consequently more frequent picking up of the bivalents of this type.

ii) Interstitial junction. The probability that the three regions, A, y and B, all open out in the same plane, is calculated as $\left(\frac{1}{3}\right)^3\times 3$, that is, $\frac{1}{9}$. This is a case of non-formation of chiasmata, and in the remaining cases of $\frac{8}{9}$ either one chiasma or two are expected to occur. The formation of one chiasma is expected either from the opening-out of A and y in the same plane and of B in a different plane, or from that of y and B in the same plane and of A in a different plane. Thus the chiasma is formed at the border of y and

B in the former case and at the border of A and y in the latter. In each case its occurrence is calculated as $3\left(\frac{1}{3}\times\frac{1}{3}\times\frac{2}{3}\right)$, that is, $\frac{2}{9}$. Therefore pairs of arms of $\frac{4}{9}$ are expected to have one chiasma in the y region. The formation of two chiasmata in this region, namely, at both the borders of A and y and of y and B, will result either from the opening-out of A and B in the same plane and of y in a different plane or from that of all these three regions in different planes. In each case its occurrence is likewise calculated as $\frac{2}{9}$. Therefore cases of $\frac{4}{9}$ must have two chiasmata. Accordingly pairs of arms having such one interstitial chiasma (−) and those having two contiguous chiasmata (+) are expected to occur in a 1:1 ratio. The records from various bivalents selected at random showed: $\overset{+}{19}\ \overset{-}{20}$. Though the number of observations is very small, owing to rather low frequency of interstitial chiasmata in this material, the data accord well with the expectation.

iii) Terminal junction. To this case also the same principles based on the two-plane theory can be applied. Cases of chiasma formation (+) at the boundary of the two regions, B and z, are expected to be twice as many as those having no chiasma (−). The former results in a characteristic double-crossed figure in which the pair of small limbs lies symmetrically within the common matrix (Fig. 3e). In the latter the chromatids come to show the end-to-end association, their free separation being arrested by the materical substance (Figs. 2 and 4).[1] Records of these two types indicated: $\overset{+}{26}\ \overset{-}{19}$. Thus the observed ratio per 3 is 1.73:1.27 for the + class and the − one, the deviation form the expected 2:1 ratio being ± 0.27. This deficiency of the + type may be attributed to greater ease in the judgment of the − type. In fact there is a certain degree of variation in the length of the open limbs, as in the proximal and interstitial junctions, and some cases were recorded in which the open limbs protrude slightly beyond the limit of the chromosome contour. These might have been such of terminal origin as described above but were excluded from the + type in recording, by reason of ambiguity in demarcation of such one from the true interstitial chiasma type.

1) The establishment of these two types of terminal chiasmata, viz., true terminal and apparent terminal, is further justified by the finding that the relation of them to the mechanism of chromonema spiralisation is entirely different from each other (see No. 15 of this series).

The configurations of bivalents can therefore be given in terms of these variants, x, y and z. For instance, one arm pair in the bivalent represented in Fig. 4 may be shown as being of the constitution of x (+). y (+). z (−). Were there no interrelation of one situation to others, the eight possible combinations of xyz, viz., +++, +−+, ++−, +−−, −++, −−+, −+−, and −−−, can be expected to occur in the ratio of 4:4:2:2:2:2:1:1. In the present study however the paucity of data did not allow to verify this point.

From the above considerations it may be said that it is possible to distinguish two types of chiasmata with respect to their mode of origin. The first may be called "*primary chiasmata*" because they are related directly to the development of interstitial repulsion. The second will be referred to as "*secondary chiasmata*"; they are controlled by the relation of the strength of the repulsion force to the surface tension and the viscous resistance set up by the matrix substance. Thus the physical nature of the matrix at the time of chromatid opening-out is to have an important bearing upon the formation of such secondary chiasmata. Since the secondary chiasma is thus subjected to two sources of variation, viz., (i) repulsion and (ii) matrical resistance, a certain variation in the length of the secondary loops may be expected under different conditions. In cases when the chromatids open out at a time when the matrix is in a less viscous and perhaps less elastic state such secondary loops may be much smaller. In preparations on which the third paper of this series was based, no such remarkable loops were noted; most probably this may bespeak such a sort of difference in the nature of the matrix between the material employed at that time and that in the present observations. This assumption is supported by the fact that even in the same anther different pollen mother cells may differ slightly in the length of secondary loops, as will be seen when one compares Fig. 2 with Fig. 4. When the repulsion force overcomes the matrical resistance, the chromatids will open out to the final point, that is, the kinetochore. The precocious type of bivalents (cf. No. 5 of this series) will be taken as affording the indisputable case of this condition.

It is evident that the present findings and interpretations are at variance with the following hypotheses of Darlington and his school, (i) of chiasma terminalisation, (ii) of chiasma interference in relation to crossing over interference and (iii) of terminal affinity (cf. Darlington '37). Full discussion on these subjects will be presented together with additional data on another occasion.

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Variations in Chromosomal Behavior During Meiosis Among Plants of *Lolium perenne* L.

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In recent years an extensive literature has been published on major irregularities in mitosis and meiosis, particularly the latter. This literature includes papers by Beadle (1929, 1930, 1931, 1932a, b, c, 1933, and 1937), Beadle and McClintock (1928), Powers and Dahl (1937), and Clark (1939) on maize, Bergner, Carteledge, and Blakeslee (1934) on Datura, Clausen (1931) on *Nicotiana Tabacum* L., Upcott (1937) and Faberge (1937) on *Lathyrus*, Darlington and Thomas (1937) on a *Festuca-Lolium* derivative, Catcheside (1939) on *Oenothera*, and others. The literature dealing with abnormalities of chromosomal behavior in hybrids and in normal plants which had been treated with various external agencies, especially radiation, is even more extensive.

On the other hand, relatively little information has been published on either the variations in minor chromosomal irregularities among supposedly normal plants of a species, or the relationship of these irregularities in different stages of the meiotic divisions. Nevertheless, a knowledge of the extent of occurrence of such irregularities which result in chromatin loss or in unequal chromo-

1) Contribution No. 15 of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Northeastern States.

somal distribution to the spores is of considerable importance. In studies of meiosis in *Triticum aestivum* L., Powers (1932a, b, and c) found variation among different plants in the frequency of non-orientated bivalents and of univalents at metaphase I and in chromatin loss. He found also that the frequency of these aberrations in the parent plants was correlated with the coefficients of variability of their progenies for weight of seed per plant, height of plant, and percentage of fruitfulness. Later, Myers and Powers (1938) demonstrated for the same species that differences in chromatin loss, as measured by percentage of immature microspores with micronuclei, were conditioned by heritable factors. Myers and Hill (1940 a and b) found variation in chromosomal irregularities during meiosis in different plants of three naturally occurring autotetraploid grasses and showed relationships between the irregularities occurring in different stages of meiosis.

The cytology of *Lolium perenne* L. has received comparatively little attention. Evans (1926), Faworski (1927), Nilsson (1933) and Peto (1933) reported the somatic number of 14 chromosomes. Peto (1933) studied meiotic behavior in two plants of *L. perenne* and one F_1 plant of *L. perenne* L.×*L. perenne* L. var. *multiflorum* (Lam.) Parnell. At metaphase I, all chromosomes were associated as bivalents. The chiasma frequencies of the three plants were 1.81, 1.62, and 1.59 per bivalent, respectively.

Materials and Methods

Plants were grown in the greenhouse during the winter of 1938–39 from commercial seed of *Lolium perenne* L., obtained from Forest Grove, Oregon. The occurrence of some weakly perennial plants and of plants with awns among the individuals obtained from this seed lot suggested some intervarietal crossing between *L. perenne* L. and *L. perenne* L. var. *multiflorum* Parnell. However, for these studies only plants of the *L. perenne* type were used. Meiotic divisions in the microsporocytes of nineteen of these plants were examined for different types of irregularities. The cytological material was fixed for about 24 hours in acetic-alcohol (one part glacial acetic acid to three parts absolute alcohol). All date were recorded and the photomicrographs made from fresh aceto-carmine smear slides.

Determinations of percentage of normal appearing pollen were made for eleven of the plants which were examined cytologically. Immediately after dehiscence, pollen was shaken onto a dry slide, a small drop of aceto-carmine was added, a cover glass was applied,

and the preparation was sealed with paraffin-gum mastic. After the preparation had stood for a few hours to allow the pollen to become well stained, counts of normal and abnormal appearing grains were made.

In determining the significance of the differences between plants, Fisher's (1936) analysis of variance was used and values of F obtained were compared with values of F for P of 0.01 and 0.05 in Snedecor's Table XXXV (1934). The statistical significance of the correlation coefficients was determined by use of Fisher's (1936) table VA.

Experimental Results

Metaphase I: A majority of the bivalents at metaphase I had two chiasmata, one in each arm, although some bivalents were observed with as many as four chiasmata. The maximum number observed in any chromosome arm was two and, where two occurred, one was usually terminal. When one chiasma occurred in a chromosome arm it was either sub-terminal or terminal. In most of the plants, the majority of the sporocytes had one or more open bivalents, i.e., bivalents with chiasmata in only one arm. These open bivalents usually had one chiasma but those with two were not uncommon. Open and closed bivalents with different numbers of chiasmata are shown in figures 1 and 2.

Since the chromosome pairs could not be separately identified at metaphase I, data on chiasma frequency (table 1) were recorded on the basis of the entire sporocyte. Results were recorded only for those sporocytes in which the number of chiasmata could be determined with certainty. The mean total chiasma frequency per plant ranged from 10.5 to 14.79, the mean freqency of terminal chiasmata varied from 8.75 to 12.4, the terminalization coefficient varied from 0.65 to 0.91, and the average number of open bivalents per sporocyte varied from 0.9 to 4.21. The data for frequencies of total and terminal chiasmata and open bivalents were analysed by the analysis of variance and the value of F for comparing mean square between plants with mean square within plants exceeded F for P of 0.01 in all three cases (table 2).

It is apparent from the statistical analysis that the plants were significantly different in total and terminal chiasma frequency and in frequency of open bivalents. Since no measure is available of variation between plants due to chance, environment, and other factors, it can not be concluded from these data that the differences observed are heritable. It is possible that the lower chiasma

frequency in some plants might have resulted from the data having been taken by chance from sporocytes at a later stage of metaphase in those plants so that part of the chiasmata already had slipped off. However, had this happened, total chiasma frequency should have been negatively correlated with the coefficient of terminalization, whereas the coefficient of correlation of these characters was –0.107 with 17 degrees of freedom, a non-significant value.

Table 1. Chiasma frequency and average number of open bivalents at metaphase I

Plant number	Number of sporocytes	Average number chiasmata		Terminalization Coefficient	Average number open bivalents
		Total	Terminal		
CT 401	31	14.5	11.4	.79	1.5
CT 405	24	10.5	8.8	.84	4.2
CT 407	35	11.1	10.1	.91	3.4
CT 410	35	13.8	11.1	.80	2.2
CT 421	25	12.5	10.5	.84	2.8
CT 423	29	14.8	12.9	.87	0.9
CT 425	32	12.1	10.8	.89	2.8
CT 431	30	10.8	9.5	.88	4.0
CT 447	26	11.9	10.4	.87	2.8
CT 456	51	12.1	9.8	.81	2.9
CT 465	17	12.9	10.0	.78	2.9
CT 478	31	13.3	12.0	.90	1.6
CT 484	28	14.3	12.0	.84	1.4
CT 493	22	14.2	12.5	.88	1.1
CT 520	24	13.0	10.6	.82	2.5
C 112-4 (3)	64	11.7	10.3	.88	3.2
C 214-4 (2)	43	12.4	9.6	.77	3.2
C 218-4 (2)	15	13.4	12.5	.93	1.5
1730	9	14.7	9.6	.65	2.8

Table 2. Summary of analysis of variance of frequency of total and terminal chiasmata and of open bivalents

Character	Between plants		Within plants		F[1]
	Mean square	DF	Mean square	DF	
Total chiasmata	48.7379	18	3.1224	552	15.61
Terminal chiasmata	36.2558	18	2.0655	552	17.55
Open bivalents	25.2103	18	1.2445	552	20.26

1) Value of F for P of 0.01 equals 2.57.

Those sporocytes for which chiasma frequencies were determined, and an additional number of metaphase I sporocytes in each plant were examined, for presence of univalents, non-orientated bivalents, and loosely attached bivalents (table 3.) In recording frequency of univalents, care was exercised to distinguish the chromosomes which were unpaired as a result of failure of chiasma formation (fig. 3) from those which had been paired normally and

then precociously disjoined. In one plant, no univalents were observed in 212 sporocytes, while sixteen plants had sporocytes with either two or four univalents in addition to normal. The range of percentage of sporocytes with univalents for the nineteen plants was zero to 9.7.

Table 3. Percentage of metaphase I sporocytes showing indicated numbers of univalents, non-orientated bivalents, and loosely attached bivalents

Plant number	Number of sporocytes	Number univalents		Number non-orientated bivalents		Number loosely attached bivalents		
		2	4	1	2	1	2	3
		%	%	%	%	%	%	%
CT 401	126	1.6	0	6.3	0	13.5	0	0
CT 405	189	2.1	0	14.8	0	16.4	0	0
CT 407	72	9.7	0	5.6	0	23.6	5.6	2.8
CT 410	165	2.4	0	4.2	1.2	7.9	1.2	0
CT 421	123	4.9	0	3.3	0	12.2	1.6	0
CT 423	199	0.5	0	3.5	0.5	1.0	0	0
CT 425	222	0.9	0.5	1.8	0	9.5	4.9	0
CT 431	72	1.4	0	4.2	0	26.4	2.8	0
CT 447	172	1.2	0	5.8	1.2	4.1	0.6	0
CT 456	300	4.7	0	5.7	0	16.7	3.0	0
CT 465	154	1.9	0	1.3	0	13.6	1.3	0
CT 478	112	2.7	0	3.6	0	9.8	4.5	0
CT 484	157	0.6	0	4.4	0	1.2	0.6	0
CT 493	122	0.8	0	13.1	1.6	3.3	0	0
CT 520	119	2.5	0	3.4	0	7.6	1.7	0
C 112-4 (3)	360	3.6	0.3	—	—	—	—	—
C 214-4 (2)	198	1.0	0	—	—	—	—	—
C 218-4 (2)	212	0	0	—	—	—	—	—
1730	68	1.5	0	—	—	—	—	—

In the majority of sporocytes, all seven bivalents were orientated on the equatorial plate with their spindle fiber attachment regions clearly stretched out towards the two poles. In a few cells in each plant, one and rarely two bivalents were lying at some distance from the plate. Examples of non-orientated bivalents are shown in figs. 4 and 5. Two plants had 14.8 and 14.7 per cent of the sporocytes showing non-orientated bivalents; the remaining thirteen plants for which this irregularity was recorded varied from 1.3 to 7.0 per cent.

Some sporocytes in all of the fifteen plants from which data were obtained showed one, two, or rarely, three loosely attached bivalents (fig. 3). These bivalents were open and had the ends of one arm attached only by a single thin strand. These cases resembled in appearance the single or imperfect chiasmata described and figured by Darlington (1929) in the Tradescantiae. However, they differ in that those found by Darlington had resulted from the breaking apart of a multiple chiasma, whereas multiple chromosomal associations which would produce multiple chiasmata were not found in this material. It is possible that these loosely attached bivalents are the

result of precocious disjunction. Whatever their nature, it will be shown later by their correlation with other irregularities that they are significant.

The correlation coefficients of chiasma frequency and degree of terminalization with percentages of metaphase I sporocytes showing univalents, non-orientated bivalents, and loosely attached bivalents are given in table 4. Percentage of univalents was negatively correlated with total chiasma frequency, the correlation coefficient exceeding the five percent point. Also the percentage of loosely attached bivalents was negatively correlated with total chiasmata and terminal chiasmata, the coefficients of correlation exceeding the one percent point in both cases. None of the other correlation coefficients was statistically significant.

Table 4. Coefficients of correlation of chiasma frequency and degree of terminalization with percentages of metaphase I sporocytes showing univalents, non-orientated bivalents, and loosely attached bivalents

Character	Percentage of metaphase I showing					
	Univalents		Non-orientated bivalents		Loosely attached bivalents	
	r	DF	r	DF	r	DF
Total chiasmata	–.458*	17	–.116	13	–.804**	13
Terminal chiasmata	–.348	17	–.074	13	–.742**	13
Terminalization coefficient	–.047	17	–.068	13	.191	13

* Exceeds five percent point.
** Exceeds one percent point.

Anaphase I: In four of the seventeen plants from which anaphase I sporocytes were examined, no irregularities in chromosomal distribution were found (fig. 6). In the other thirteen plants, some sporocytes showed one or more univalents which were lagging and undergoing an equational division (table 5). Occasionally, the chromatids of the lagging univalents already had separated and were approaching the two daughter chromosome groups (figs. 7, 8, 9 and 10). In one plant, CT 405, 41.6 percent of the anaphase I sporocytes had one or more lagging univalents (figs. 7, 8, and 9), six occurring on one of the 89 cells examined (fig. 10). In the remaining plants, the percentage of sporocytes showing laggards varied from zero to 2.5.

The origin of laggards at anaphase I is of considerable interest. Such laggards commonly can be attributed to the failure of unpaired chromosomes at metaphase I to move properly to the poles at anaphase. In this material, the correlation coefficient of metaphase

Table 5. Percentage of anaphase I sporocytes showing indicated number of lagging univalents

Plant number	Number of sporocytes	Number lagging univalents 1	2	3	4	5	6	Total
		%	%	%	%	%	%	%
CT 401	126	1.6	0	0	0	0	0	1.6
CT 405	89	13.5	22.5	3.4	1.1	0	1.1	41.6
CT 407	159	1.3	0	0.6	0	0	0	1.9
CT 410	137	0.7	0	0	0	0	0	0.7
CT 421	201	1.5	1.0	0	0	0	0	2.5
CT 423	61	0	0	0	0	0	0	0
CT 425	166	1.2	0	0	0	0	0	1.2
CT 456	112	0.9	0.9	0	0	0	0	1.8
CT 465	228	0.9	0.4	0	0	0	0	1.3
CT 478	136	0.7	0.7	0	0	0	0	1.4
CT 484	220	0	0	0	0	0	0	0
CT 493	200	0	0.5	0	0	0	0	0.5
CT 520	102	0	1.0	0	0	0	0	1.0
C 112-4 (3)	104	1.9	0	0	0	0	0	1.9
C 214-4 (2)	134	1.5	0	0	0	0	0	1.5
C 218-4 (2)	94	0	0	0	0	0	0	0
1730	38	0	0	0	0	0	0	0

I univalents with anaphase I laggards was 0.016, a non-significant value (table 6). Comparison of the data given in table 3 with those given in table 5 shows the error in this analysis. Whereas the frequency of lagging univalents was low in sixteen of the plants, it was very high in CT 405. The number of metaphase I univalents recorded in this plant was far too small to account for the number of laggards at anaphase I. By excluding data for CT 405 from the calculations, a correlation coefficient of 0.635 is obtained. This value exceeds the one percent point. Apparently, metaphase I univalents are correlated with lagging at anaphase I, but laggards are also arising from other sources, at least in CT 405. Comparison of the data in tables 3 and 5 also shows that in all plants except CT 405

Table 6. Coefficients of correlation of lagging chromosomes at anaphase I with other meiotic irregularities

Characters	MI univalents r	MI univalents DF	Non-orientated bivalents r	Non-orientated bivalents DF	Loosely attached bivalents r	Loosely attached bivalents DF	No. quartets with micronuclei r	No. quartets with micronuclei DF
	Including data from CT 405							
AI univalents	0.016	15	0.627*	11	0.189	11	0.984**	15
	Excluding data from CT 405							
AI univalents	0.635**	14	-0.218	10	0.816**	11	0.282	14

* Exceeds five percent point.
** Exceeds one percent point.

the number of laggards at anaphase I was less than the number of unpaired chromosomes at metaphase I. In sporocytes showing unpaired chromosomes at metaphase I, two or four univalents were always found, as might be expected, never one or three. On the other hand, among sporocytes showing lagging at anaphase I, those with a single laggard were most common. Furthermore, the percentage of sporocytes showing one or more lagging univalents at anaphase I was lower in most plants than the percentage of metaphase I sporocytes showing univalents. These results indicate that unpaired chromosomes frequently move to the poles with the disjoining members of the bivalents.

The relation of lagging univalents at anaphase I to non-orientated bivalents at metaphase I is not clear from these data. The correlation coefficient of these two characters is 0.627 (exceeds the one percent point) when the data from all plants are used, while it is –0.218 when the data for CT 405 are excluded from the calculations. This plant had the highest frequency of each of these irregularities. However, CT 493 had 14.7 percent of the metaphase I sporocytes showing non-orientated bivalents as compared with 14.8 percent for CT 405, whereas, in contrast to CT 405, it had only 0.5 percent of the anaphase I sporocytes showing laggards. It seems uncertain whether non-orientated bivalents have contributed appreciably to the lagging univalents at anaphase I in the plants used in these investigations.

The correlation coefficient of percentage of anaphase I sporocytes showing laggards with percentage of metaphase I sporocytes showing loosely attached bivalents was 0.189 when the data for CT 405 were included and 0.816 when they were excluded from the calculations (table 6). In this case again the high frequency of lagging univalents in CT 405 places undue emphasis in the calculations on the data from this plant, thus obscuring the relationship which appears to exist in the other plants.

In the seventeen plants in which anaphase I figures were examined, the numbers of chromosomes in the two groups at anaphase were determined in 633 sporocytes in which lagging univalents did not occur. In every case the distribution was normal, seven chromosomes passing to each pole.

Telophase I and Interphase I: Lagging chromosomes at anaphase I were observed to divide equationally, the two daughter half chromosomes separating and moving to the poles. At early telophase I, some were apparently close enough to the poles to be included in the daughter nuclei, while others were lagging too far behind. These

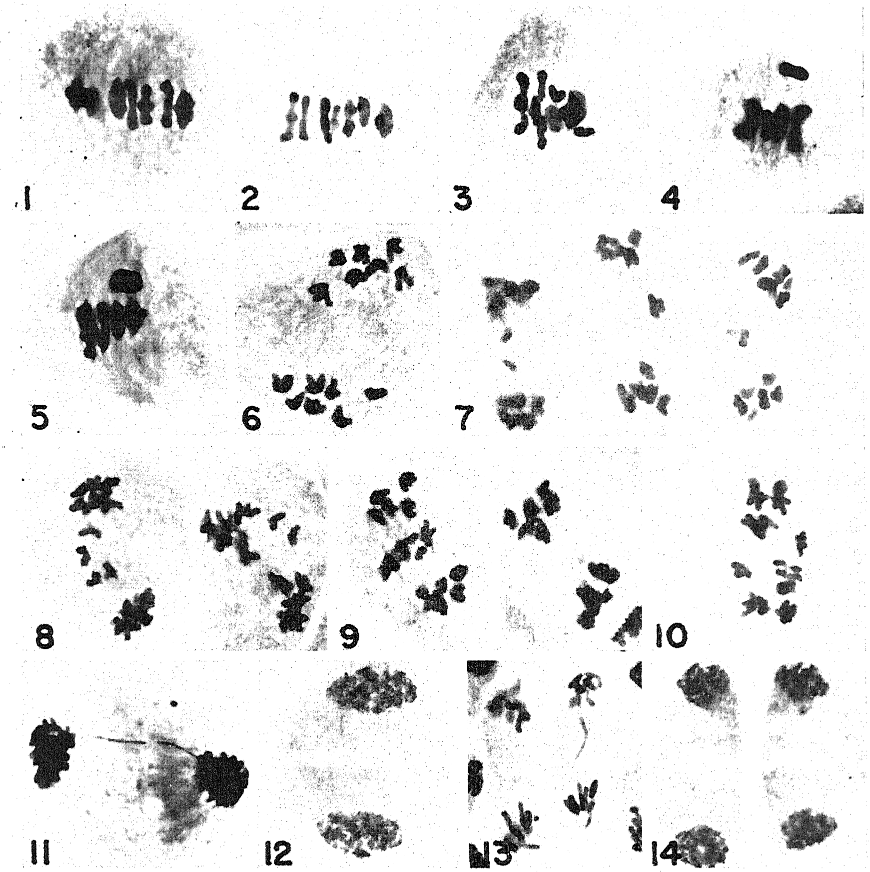
would be left in the cytoplasm to form micronuclei. At this stage, no undivided univalents were observed to be lagging. If neither chromatid from a single lagging univalent had been included in a daughter nucleus, two micronuclei, one in each cell, would be found at interphase I, while if one chromatid had been included in a daughter nucleus, one micronucleus would result. In a similar manner the types of interphase I sporocytes expected from two, three, or more lagging univalents can be determined. A normal appearing interphase I sporocyte is shown in fig. 12.

The percentage of different types of interphase I sporocytes found in each plant are shown in table 7. This table also gives the number of micronuclei obtained per 100 sporocytes and the number expected on the assumption that all daughter half chromosomes from lagging univalents formed micronuclei. The number of micronuclei obtained was less than the number expected in thirteen of the seventeen plants. In two of the plants, no micronuclei were expected and none was obtained, while in the other two plants none was expected and 0.5 and 0.9 per 100 sporocytes were obtained. In one of these plants, 1730, the 38 anaphase I figures examined was probably too small a number for a significant estimate, while in the other, CT 484, only one micronucleus was observed in 221 interphase I sporocytes so that the excess obtained over expected is probably without significance. It may be concluded that in most of the plants

Table 7. Percentage of interphase I sporocytes showing indicated number of micronuclei in each cell, the number of micronuclei expected per 100 sporocytes, and the number obtained

Plant number	Number of sporocytes	Number of micronuclei in sister cells						Number of micronuclei per 100 sporocytes	
		0–1	1–1	0–2	1–2	1–3	2–2	Obtained	Expected
		%	%	%	%	%	%	%	%
CT 401	295	0	0	0	0.3	0	0	0.9	3.2
CT 405	257	10.1	2.7	1.2	1.2	0	0.8	24.7	159.4
CT 407	210	0	0	0	0	0	0	0	6.2
CT 410	140	0	0	0	0	0	0	0	1.4
CT 421	192	2.1	0	0	0	0	0	2.1	7.0
CT 423	231	0	0	0	0	0	0	0	0
CT 425	178	1.1	0	0	0	0	0	1.1	2.4
CT 447	158	1.3	0.6	0	0	0	0	2.5	—
CT 456	206	2.0	0	0	0	0	0	2.0	5.4
CT 465	219	0.5	0	0.5	0	0	0	1.5	3.4
CT 478	200	1.0	0	0	0	0	0	1.0	4.2
CT 484	221	0.5	0	0	0	0	0	0.5	0
CT 493	158	0	0	0.6	0	0	0	1.2	2.0
CT 520	178	0	0	0	0	0	0.6	2.4	4.0
C 112–4 (3)	393	0.5	0.2	0	0	0	0	0.9	3.8
C 214–4 (2)	302	0.3	0	0	0	0	0.3	1.5	3.0
C 218–4 (2)	102	0	0	0	0	0	0	0	0
1730	107	0.9	0	0	0	0	0	0.9	0

investigated the majority of the chromatids from lagging and dividing univalents reach the poles in time to be included in the interphase I nuclei.



Figs. 1–14. ×ca. 750. 1 and 2. Normal metaphase I. 3. Metaphase I showing two univalents and a loosely attached bivalent. 4 and 5. Metaphase I with a non-orientated bivalent. 6. Normal anaphase I with seven chromosomes in each daughter group. 7. Three late anaphase I sporocytes with lagging and dividing univalents. 8. Two late anaphase I sporocytes with lagging and dividing univalents. 9. One anaphase I with four lagging and dividing univalents and one normal anaphase I. 10. Anaphase I with lagging and dividing univalents. 11. Dicentric chromatid bridge and acentric fragment at telophase I. 12. Normal interphase I. 13. Sister cells at telophase II, one showing a dicentric chromatid bridge and the other an acentric fragment. 14. Normal quartet showing planes of first and second division.

Quartets: In recording data from the quartets, only those were selected in which the planes of the first and second divisions could be determined (fig. 14). In the eighteen plants from which data were obtained, the percentage of quartets having one or more micronuclei in one or more of the four cells ranged from zero to 30.0 percent

(table 8). The coefficient of correlation between percentage of quartets having micronuclei and percentage of anaphase I sporocytes showing univalents was 0.984, a value in excess of the one percent point. A comparison of table 5 with table 8 indicates that this high value was contributed to largely by the behavior of CT 405. This

Table 8. Percentage of various types of quartets and the total percentage of quartets showing micronuclei

Plant number	Number of sporocytes	Types of quartets[1)]											Percent with micronuclei
		1	2	3	4	5	8	9	11	12	22	30	
		%	%	%	%	%	%	%	%	%	%	%	
CT401	131	0	0	0	0	0	0	0.8	0	0	0	0	0.8
CT405	110	12.7	6.4	4.5	0	0	1.8	1.8	0.9	0.9	0.9	0	30.0
CT407	231	0	0	1.7	0	0	0.4	0.4	0	0	0	0	2.6
CT410	166	0	0	0.6	0	0	0	0	0	0	0	0	0.6
CT421	274	0	0.4	0.4	0.4	0	0	0	0	0	0	0	1.1
CT423	267	0.4	0	0.8	0	0	0	0	0	0	0	0	1.1
CT425	315	1.0	0.3	2.9	0	0	0.3	0	0	0.3	0	0	4.8
CT447	290	0.3	0	1.0	0	0	0.3	0.3	0	0	0	0	2.1
CT456	262	0	0	0.8	1.1	0.4	0.4	0	0	0.4	0	0.4	3.4
CT465	303	0	0.3	0.3	0	0	0	0.3	0	0	0	0	1.0
CT478	204	0	0	1.0	0.5	0	0	0	0	0	0	0	1.5
CT484	203	0.5	0	0	0	0	0	0	0	0	0	0	0.5
CT493	200	0	0	0	0	0	0	0.5	0	0	0	0	0.5
CT520	207	0.5	0.5	1.0	0	0.5	0	0.5	0	0	0	0	3.0
C112-4(3)	181	0	0	0	0	0	0	0.6	0	0	0	0	0.6
C214-4(2)	252	0.4	0	0	0	0	0	0	0	0	0	0	0.4
C218-4(2)	300	0	0	0	0	0	0	0	0	0	0	0	0
1730	70	1.4	0	0	0	0	0	0	0	0	0	0	1.4

1) Types of quartets are shown in fig. 15.

plant had 41.6 percent of its anaphase I sporocytes with lagging univalents and 30.0 percent of its quartets with micronuclei. Excluding data for CT 405 from the calculations, the correlation coefficient becomes 0.282, a nonsignificant value. On the basis of observations throughout meiosis, there is little question but that lagging and dividing univalents at anaphase I do form micronuclei in the quartets either through failure of the daughter half chromosomes to reach the poles in the first division or their inability to move normally in the second division. The absence of a significant correlation coefficient in the sixteen plants (excluding CT 405) may have resulted from at least three factors, (1) lack of significant variation in one variable or the other, (2) differential frequency of the inclusion of daughter half chromosomes in the microspore nuclei in different plants, or (3) the origin in some plants at least of micronuclei from some source in addition to laggards at anaphase I. No estimate of error is available from these data for determining the significance of the differences between plants for either anaphase I laggards or presence of micronuclei. The statistically significant

correlation coefficients between lagging univalents at anaphase I and other characters (table 6) indicate that the differences between plants for this character were statistically significant.

In table 9 are shown the number of micronuclei obtained per 100 quartets and the number calculated on the assumption that all daughter half chromosomes from lagging univalents at anaphase I formed micronuclei. In ten of the plants, the number of micronuclei obtained varied from 26.7 to 71.4 percent of the calculated. In these plants, apparently a considerable proportion of the half chromosomes were included in the microspore nuclei. One plant had no micronuclei in the 300 quartets examined and none was expected, since no laggards were found in 94 anaphase I sporocytes. In the remaining six plants the obtained number exceeded the calculated. In one plant, CT 425, almost three times as many micronuclei were obtained as expected. These excesses of obtained over calculated suggest the possibility that micronuclei were produced in these plants from some source in addition to anaphase I laggards. Further evidence in support of this may be obtained from a comparison of types of quartets obtained with those expected on the assumption that all micronuclei result from the loss of daughter half chromosomes from lagging anaphase I univalents. The types of quartets, based on number and position of micronuclei, have been calculated by Myers and Hill (1940b) for sporocytes with one, two, and three laggards. These types are shown in fig. 15 and the percentages of the different types in each plant are given in table 8. Unexpected types of quartets were found in seven plants, in five of which an excess of obtained over calculated numbers of micronuclei was obtained. Three of these five had no laggards at anaphase I and no micronuclei were expected; the other two plants, CT 425 and CT 456, had 0.6 and 0.4 percent, respectively, of unexpected types of quartets. Two plants, CT 401 and C 112–4 (3), which had fewer micronuclei than calculated, had 0.8 and 0.6 percent of unexpected types of quartets,

Table 9. Relationship of the number of micronuclei obtained per 100 quartets to the number expected on the assumption that all half chromosomes from lagging anaphase I univalents formed micronuclei

Plant number	Number of micronuclei per 100 quartets		o/e×100
	Obtained	Expected	
CT401	1.6	3.2	50.0
CT405	59.8	159.4	37.5
CT407	3.7	6.2	59.7
CT410	0.6	1.4	42.8
CT421	2.8	7.0	40.0
CT423	1.6	0	—
CT425	7.0	2.4	291.7
CT456	11.2	5.4	207.4
CT465	1.5	3.4	44.1
CT478	3.0	4.2	71.4
CT484	1.0	0	—
CT493	1.0	2.0	50.0
CT520	6.5	4.0	162.5
C 112–4 (3)	1.2	3.8	31.6
C 214–4 (2)	0.8	3.0	26.7
C 218–4 (2)	0	0	—
1730	2.8	0	—

respectively. The percentages of unexpected quartet types are in all plants so small that they would have no significance in themselves, but their occurrence tends to support the evidence from the excess of obtained over calculated number of micronuclei in indicating that micronuclei arose in certain plants from sources in addition to loss of daughter half chromosomes from anaphase I laggards.

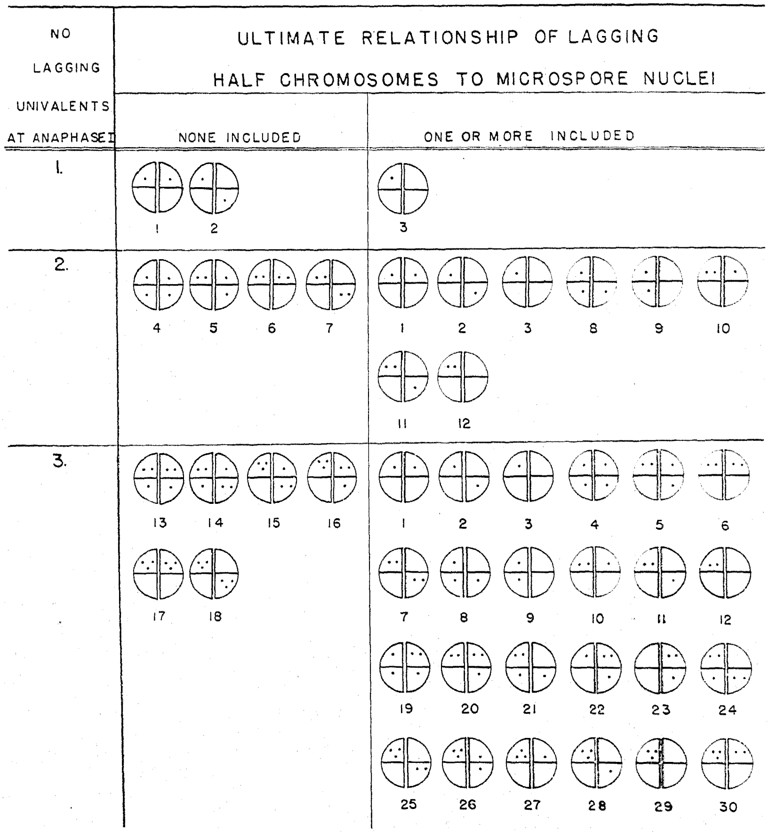


Fig. 15. Types of quartets expected from sporocytes with 1, 2 and 3 lagging univalents at anaphase I if micronuclei in the quartets result only from the loss of daughter half chromosomes of the anaphase I laggards.

Dicentric Chromatids and Acentric Fragments: The percentage of sporocytes with dicentric bridges and acentric fragments at anaphase I and the percentage with chromatin bridges at interphase I and in the quartets are shown for each plant in table 10. Ten of the seventeen plants had from 0.6 to 7.1 of the anaphase I sporocytes with dicentric chromatid bridges and acentric fragments. In one plant one of the chromatin bridges consisted of two dicentric chromatids. In CT 407 no sporocytes were found with chromatin bridges but one out of 159 had an acentric fragment. The remaining six plants had

neither dicentric bridges nor acentric fragments. Of the ten plants with chromatin bridges and fragments at anaphase I, seven also had interphase I sporocytes with chromatin bridges. In most cases the bridge was accompanied by a fragment lying in the cytoplasm but occasionally no fragment was visible, perhaps because of its inclusion in one of the interphase nuclei or disintegration in the cytoplasm. Fig. 11 shows a sporocyte at telophase I with a bridge and fragment. The absence of bridges at interphase I in the other three plants which had bridges at anaphase I may have been due to chance or to difficulties of interpretation. In most cases at interphase I, the chromatin bridge had broken and frequently only slight protrusions from the interphase I nuclei remained. Some such cases may have been overlooked.

Table 10. The number of sporocytes examined and the percentage showing dicentric bridges and acentric fragments at different stages of meiosis

Plant number	Anaphase I			Interphase I		Quartets	
	Number counted	Bridge and fragment	Fragment	Number counted	Bridge[1]	Number counted	Bridge[1]
		%	%		%		%
CT401	126	7.1	0.0	295	1.4	131	0.8
CT403	—	—	—	204	0.5	213	0.5
CT405	89	3.4	0.0	257	1.6	110	0.0
CT407	159	0.0	0.6	210	0.0	231	0.4
CT410	137	1.4	0.0	140	0.0	166	0.0
CT421	201	1.0	0.0	192	0.5	274	0.4
CT423	61	6.6	0.0	231	0.0	267	0.0
CT425	166	0.6	0.6	178	1.7	315	0.0
CT447	—	—	—	158	2.5	290	0.0
CT456	112	2.7	0.0	206	4.0	262	1.1
CT465	228	0.0	0.0	219	0.0	303	0.0
CT478	136	0.0	0.0	200	0.0	204	0.0
CT484	220	1.8	0.0	221	0.9	203	1.0
CT493	200	0.0	0.0	158	0.0	200	1.0
CT520	102	0.0	0.0	178	0.0	207	0.0
C 112–4 (3)	104	0.0	0.0	393	0.0	181	0.0
C 214–4 (2)	134	1.5[2]	0.0	302	0.0	252	0.4
C 218–4 (2)	94	3.2	0.0	102	1.0	300	0.0
1730	38	0.0	0.0	107	0.0	70	0.0

1) Bridges frequently were accompanied by a fragment.
2) One of the chromatin bridges consisted of two chromatids.

It has been demonstrated by McClintock (1933, 1938) and others that single crossovers in heterozygous inverted areas which do not contain the spindle fiber attachment region will result in dicentric bridges and acentric fragments like those observed at anaphase I and telophase I in this material. A four-strand double crossover within the inverted area would result in a double bridge with two fragments such as the one found in C 214–4(2). A single crossover within the inverted area occurring at the same time as a

single crossover between the inversion and the spindle fiber attachment (see McClintock, 1938) would result in a fragment but no bridge at anaphase I, both spindle fiber attachments of the dicentric chromatid passing to the same pole. Such sporocytes with fragments but not bridges were recorded in plants CT 407 and CT 425. Anaphase II of such sporocytes usually should have a chromatin bridge in one cell and a fragment in one cell (fig. 13). Consequently, such sporocytes usually should form quartets with a chromatin bridge between the sister nuclei from one of second divisions. The fragment might or might not be visible depending upon whether it had been included in a microspore nucleus (McClintock, 1938). Chromatin bridges were found in the quartets of seven of the twelve plants in which bridges occurred at anaphase I, interphase I, or both. Also a chromatin bridge was found in one quartet in CT 407 which had a fragment but no bridge in one anaphase I sporocyte. The results suggest that thirteen of the nineteen plants were heterozygous for inversions.

Evidence of Irregularities in Pre-Meiotic Mitoses: On the 225 metaphase I sporocytes examined in CT 425, three were aneuploid, while all others had fourteen chromosomes. Of these, one had eight bivalents, one had seven bivalents plus one univalent plus one fragment, and one had seven bivalents plus two fragments. In CT 484, 158 metaphase I sporocytes were studied and one was tetraploid, while the others were diploid.

Appearance of the Pollen: The data on percentages of normal and abnormal appearing pollen are shown in table 11. The grains which were classed as small were plump, with normal-appearing cytoplasm and three normal appearing nuclei, their only apparent deviation

Table 11. Percentages of normal and different types of abnormal appearing pollen produced by different plants

Plant number	Number examined	Types of pollen						
		Normal	Small	Empty	Trace of cytoplasm	One nucleus	Two nuclei	3 nuclei deficient cytoplasm
		%	%	%	%	%	%	%
CT 401	232	94.8	0.0	4.3	0.9	0.0	0.0	0.0
CT 405	254	65.8	7.9	15.8	1.6	2.0	0.0	7.1
CT 407	304	95.7	0.0	4.3	0.0	0.0	0.0	0.0
CT 421	308	35.7	11.4	34.4	11.4	1.0	3.6	2.6
CT 423	303	93.4	1.0	4.0	0.0	0.0	1.6	0.0
CT 425	207	68.1	11.1	3.9	4.4	4.4	5.8	2.4
CT 447	337	34.7	0.0	15.4	1.8	20.5	22.0	5.6
CT 456	311	74.0	5.8	18.0	0.3	1.3	0.0	0.6
CT 484	245	42.0	17.6	18.0	3.3	5.7	9.0	4.5
CT 493	548	39.0	14.0	23.2	8.0	5.1	7.8	2.7
CT 520	198	21.2	8.6	23.7	20.7	13.1	8.6	4.0

from normal being their small size. The other classes were more clearly abnormal. Those grains with a trace of cytoplasm were apparently in a late stage of disintegration, the grains were shriveled, a small amount of cytoplasm remained usually near one side, and no nuclei were seen. The grains with one or two nuclei usually were smaller, somewhat shriveled and with the cytoplasm reduced so that it did not fill the entire grain. Finally some grains were seen with three nuclei, but a reduced amount of cytoplasm which did not stain normally. It was thought that these grains were beginning to disintegrate.

The percentage of normal pollen grains in the eleven plants varied from 21.2 to 95.7 percent. A comparison of these data with the data on chromosomal behavior in the same plants reveals little or no relationship between the degree of aberrant chromosomal behavior and percentage of normal appearing pollen. Plant CT 405 showed much greater percentages of laggards at anaphase I and chromatin loss at interphase I and in the quartets than any other plant. Yet five of the plants had less normal pollen than CT 405. There seems to be little question that chromosomal irregularities, particularly those resulting in chromatin loss, would cause the production of inviable gametes and, consequently, abnormal appearing pollen in a diploid plant. Apparently other factors, genetical, cytological, or both, were causing a considerable amount of pollen abortion in some of these plants, thus preventing the relationship between meiotic irregularities and pollen abortion from becoming apparent.

Summary

1. Statistically significant differences were found among nineteen plants of *Lolium perenne* L. in total number of chiasmata, number of terminal chiasmata, and number of open bivalents per microsporocyte. The chiasma frequency was not correlated with the terminalization coefficient.

2. The percentage of metaphase I sporocytes showing univalents varied from zero to 9.7 in the different plants. The range in percentage of metaphase I sporocytes showing non-orientated bivalent and loosely attached bivalents was 1.3 to 14.8 percent and 1.0 to 32.0 percent, respectively.

3. Statistically significant negative correlation coefficients were obtained (1) between total chiasma frequency and percentage of metaphase I sporocytes having univalents and (2) between both total and terminal chiasma frequency and percentage of sporocytes having loosely attached bivalents. Percentage of sporocytes with

non-orientated bivalents was not correlated with chiasma frequency. The terminalization coefficient was not correlated with metaphase I univalents, non-orientated bivalents, or loosely attached bivalents.

4. In one plant, CT 405, 41.6 percent of the anaphase I sporocytes had one or more lagging univalents, a maximum of six occurring in one sporocyte. In the remaining plants, the percentage of sporocytes showing laggards varied from zero to 2.5. The lagging univalents divided equationally in all observed cases.

5. The percentages of metaphase I sporocytes with univalents and with loosely attached bivalents were correlated with frequency of lagging univalents at anaphase I when the data for CT 405 were not included in the calculations. Apparently most of the laggards in this plant arose from some source other than these two.

6. The numbers of chromosomes were determined in the two groups at anaphase I in 633 sporocytes in which lagging chromosomes did not occur and in all cases seven chromosomes were seen in each group.

7. The data on frequency of micronuclei in the sporocytes at interphase I indicated that a majority of the daughter half chromosomes from anaphase I laggards were included in the daughter nuclei.

8. The percentage of quartets with one or more micronuclei in one or more of the four cells ranged from zero to 30.0 percent in eighteen plants. Apparently lagging and dividing univalents at anaphase I were important in producing micronuclei in the quartets both as a result of failure of the daughter half chromosomes to reach the poles in the first division and their inability to move normally in the second division.

9. In ten of the plants, the number of micronuclei per 100 quartets varied from 26.7 to 71.4 percent of the expected number calculated on the assumption that all daughter half chromosomes from anaphase I laggards formed micronuclei. No micronuclei were expected in one plant and none was obtained. In the remaining six plants, the number of micronuclei exceeded the calculated. One plant had about three times as many as expected. These results suggested that micronuclei were produced in certain plants from some source in addition to lagging univalents at anaphase I.

10. This conclusion was supported by the occurrence in seven plants of types of quartets, based on number and position of micronuclei, which were not expected on the assumption that all micronuclei were formed from daughter half chromosomes derived from lagging and dividing univalents at anaphase I.

11. The presence of dicentric bridges and acentric fragments

at anaphase I, interphase I, and in the quartets indicated that thirteen of the nineteen plants were heterozygous for inversions.

12. Rare aneuploid sporocytes in one plant and a tetraploid sporocyte in another plant suggested occasional irregularities in premeiotic divisions.

13. Percentage of normal appearing pollen varied from 21.2 to 95.7 percent in eleven plants. Apparently factors in addition to the meiotic irregularities studied were conditioning pollen abortion in the plants.

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Chromosome Studies on *Trillium kamtschaticum* Pall. XV. A contribution to the present status of knowledge on the mechanism of chromonema coiling[1)]

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A priori one can assume that the formation of a cylindrical spiral by an untwisted thread takes place under one of two conditions, either (i) by rotation of its ends or (ii) by twisting the thread in the opposite direction to the spiral assumed and thus without any rotation of the ends (cf. Darlington '35). In the first possibility, the thread itself is free from any internal twisting (Kuwada's "orthospiral", '39) and when the thread is divided into two along its axis, the daughter threads take the form of the relational spiral system and are inseparable from each other, unless some certain special mechanism of separation is assumed; in the second case, the thread is twisted (Kuwada's "anorthospiral" '39) but when divided along the plane of the twisting the resulting threads constitute a parallel spiral system and are separable from each other without any entangling (cf. No. 12 of this series)[2)].

Which of these two ways of spiral formation then is applicable to the case of the chromonema? The writer's previous findings on the structure of meiotic chromosome spirals in *Trillium kamtschaticum* seem to point to the validity of the first possibility (see under Discussion). Contrary to this view, Darlington ('35) denies the first possibility from the reasons that if one accepts this view of the method of spiral formation (i) it would be difficult to understand how in chromosomes or chromosome parts whose ends are both mechanically unable to rotate, such as in ring-shaped

1) Aided by a grant from the Japan Society for the Advancement of Cytology.

2) To these two categories of the double-thread spiral, Kuwada ('39) applies different terms: "double-stranded" and "compound" spirals in the place of the present terms, "relational" and "parallel" spirals respectively. Kuwada uses the term "relational" when the strands of his double-stranded spiral are separated from each other and take an appearance of two intertwining spirals, but since such a configuration is not essentially different from the ordinary spiral, it will be convenient to cover his double-stranded spiral and its derivatives under the term relational spiral. Darlington's original usage of relational spiral for prophasic chromatid interlacing may be distinguished, if necessary, as "relational twisting."

chromosomes or chiasma loops, the normal contraction can take place, and furthermore (ii) it would be then expected that longer chromosomes contract more slowly than shorter ones, while such a supposition is not in accord with the actual facts. This second objection, however, does not seem of prime importance, because if one assumes that the internal twisting occurs first in the thread itself and then a spiral is assumed by it *in the same direction* as the twists, as described by Kuwada ('37), the first possibility will be equal to the second in the procedure of spiralling. Then the first objection made by Darlington must be here considered.

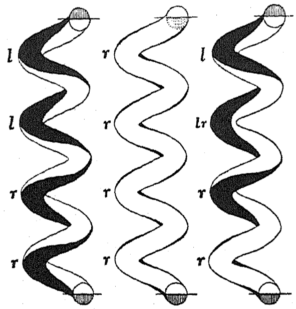


Fig. 1. Three models of coiling of a thread whose ends are fixed. *a*, the balanced form, coils in one direction being compensated by coils in the opposite direction (dextrorse turns being equal in number to sinistrorse ones) and the thread being completely free from twisting. *b*, the unbalanced form, the spiral being compensated by the internal twisting in the opposite direction. *c*, an example of the mixed type. *l* and *r* represent sinistrorse and dextrorse coils respectively. When in these models the black and the blank parts are regarded as representing each a single thread, the balanced form constitutes a relational double-thread spiral and the unbalanced form a parallel one.

Let it be assumed that the thread is untwisted throughout the entire length before spiralisation begins and both its ends are fixed, not permitting rotation. Then one can expect the following three kinds of configurations of spiral to be assumed by such a thread.

(i) The twists brought out by a spiral in one direction are cancelled out by the formation of the corresponding spiral in the opposite direction (Fig. 1a). This is the case in plant tendrils (Kuwada ('37). The thread itself remains completely untwisted, and hence even if its end or ends were released, such a spiral will remain intact. In this sense this spiral may be called a "balanced" type.

(ii) The thread is twisted in the opposite direction to the spiral assumed as many times as the gyre number (Fig. 1b). Thus the twists brought out by the spiral are compensated by the twisting of the thread itself in the opposite direction. In this form, when its end or ends set free, the thread will tend to untwist by rotating. In this sense this form may be referred to as an "unbalanced" type.[1)]

1) The terms used here "balanced" and "unbalanced" should not be confused with those by Abraham ('39); his *balanced* and *unbalanced spirals* are synonymous with Kuwada's *anortho-* and *orthospirals* respectively. It must be noted that the writer's present terminology refers only to the spiral of a thread both ends of which are fixed.

(iii) The above two kinds of coling occur at the same time within a single thread (Fig. 1c). Thus the resultant spiral is partly balanced and partly unbalanced and may be called a mixed type. The proportion of dextrorse turns and sinistrore ones in this type of spiral differs according to different proportions of the balanced and the unbalanced spirals; in any case however the following relationship must be fulfilled: $Gr + Tr = Gl + Tl$, where Gr and Gl represent dextrorse and sinistrorse gyre numbers, respectively, of the spiral and Tr and Tl dextrorse and sinistrorse twist numbers, respectively, of the thread.

The writer's previous studies on the direction of coiling (Nos. 3 and 4 of this series) were concerned with chromosomes with their distal ends free. Now in comparison with these, a study must be done on chromonemata with both ends fixed. Such sources of material may be sought in the following cases of bivalents, (i) chiasma loops, (ii) bridges and (iii) inversion regions. In the present study the first two cases were utilizable.

In this way of investigation it is hoped that one may touch the problem whether at the beginning stage of opening-out the paired chromatids are already in a twisted state as some workers have assumed (e. g. Darlington '35, Atwood '37, Kuwada '39) or are entirely untwisted as the writer's Neo-two-plane theory demands, and in this connection some light may be thrown upon the mechanism of chromonema coiling.

Observations

i) Chiasma loops

In the present study on chiasma loops the same preparations as in the previous paper (No. 14 of this series) were mainly employed. In that paper it was pointed out that there are distinguished two kinds of so-called terminal chiasma: the true terminal chiasma and the apparent terminal one which is in fact of an extremely subterminal nature. Observations on coiling structure in the chiasma loops were then made under the following three cases: (i) true terminal (ii) apparent terminal and (iii) interstitial. It must be recalled here that the paired kinetochores in a bivalent serve as a fixed point during the spiralisation process and hence it follows that the chiasma loops referred to here are those including loops between the kinetochore and an adjacent chiasma as well as loops between two successive chiasmata.

Regarding the structure of these loops, one of the most char-

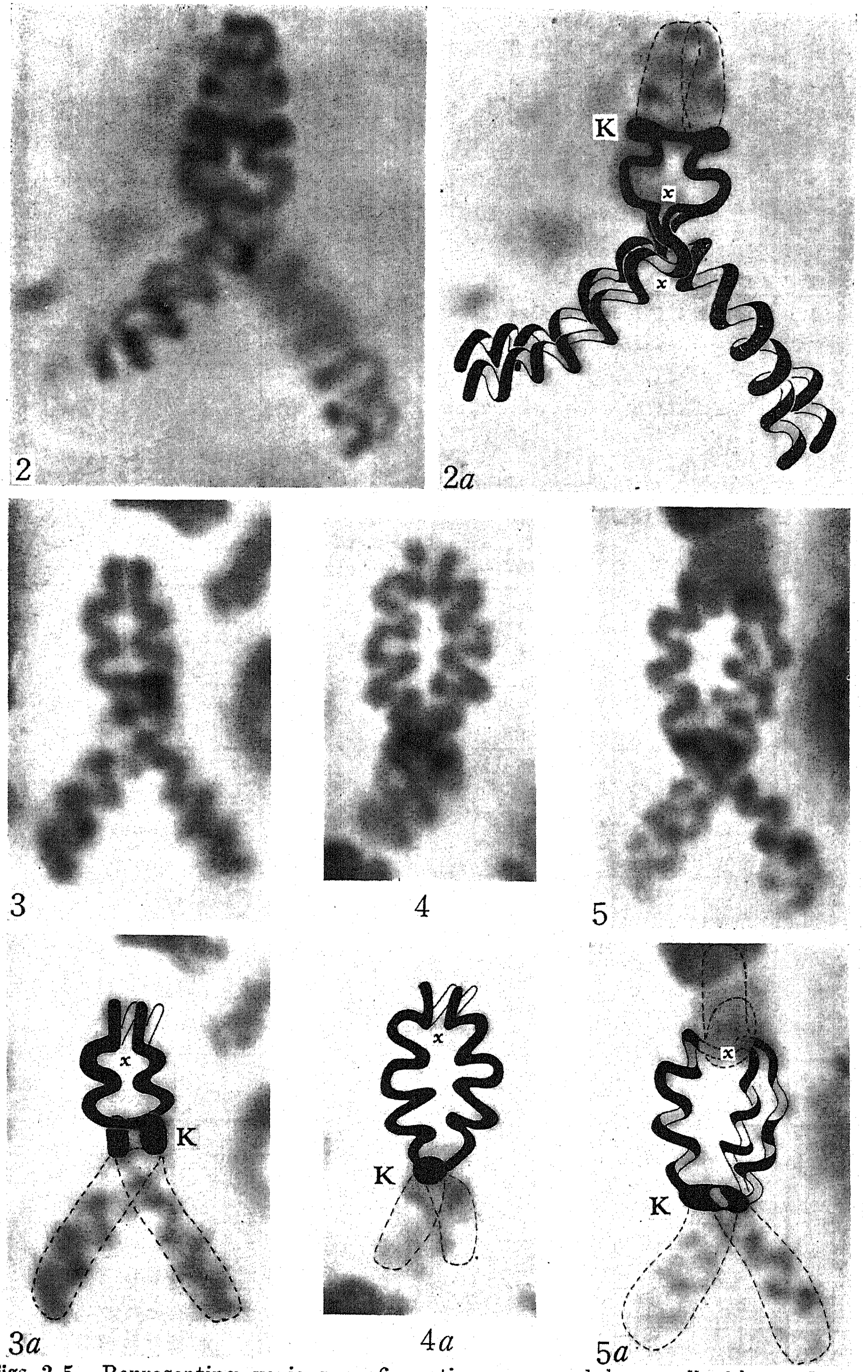
acteristic features is that when the loops are small and below a certain limit in length, they can not usually take the regular cylindrical form of spiral. This limit of length seems to be about four coil lengths and within this limit the paired chromatids are usually subjected to mere corrugation, though it was often difficult to distinguish this from an incomplete spiral, because the corrugation is defined as representing a wavy condition of a thread lying on an Euclidean plane and the incomplete spiral is a transition form to the regular cylindrical spiral, and these two configurations are in so delicate relationship that the corrugation is easily transformed to an incomplete spiral.

It is a noteworthy fact that such a corrugation usually occurs in a symmetrical form in the corresponding arms of a loop. These corrugation figures in loops of about one, two and three coil lengths are shown, respectively, in Figs. 2, 3 and 4. This limitation in length is however variable to a certain extent; the writer met with only one case in which the interstitial loop-arms of three coil lengths take a regular spiral (cf. Table 3). It will be noticed here that similar corrugated configurations are clearly shown in Fig. 11 of paper No. 11 of this series which represents a metaphase I plate of *Lilium Hansoni*.

Furthermore in cases where the loops are extremely small, probably less than one coil length such as in secondary chiasma loops, the loop-arms seem to be highly resistant even to corrugation and usually appear as almost straight lines (see Figs. 7–9). This principle holds true even when the distal ends of such loops are free as represented in Figs. 3 and 4 and others. In such small loops, the individual chromatids lie parallel and are clearly discernible from each other even when the other part takes a fused spiral form and does not show its duality.

The incapability of such small loops to take a regular spiral form seems to result in a certain stress within the paired chromatids and sometimes one meets with cases where the paired chromatids in such loops coil independently (at least in part) of each other as illustrated by the right arm in Fig. 5, a condition which never occurs in free arms.

Beyond this limit of length, the chromonemata can take a regular spiral. The general appearance of the spiral is the same as in free arms, though there occurs frequently a certain distortion in the spiral pitch as indicated by the arrows in Figs. 7 and 8. As seen in these figures this distortion occurs near by the chiasma at a place where the spiral is acute-angledly bent. Since no such distortion is met with in free arms, this is undoubtedly a strain



Figs. 2–5. Representing various configurations assumed by small chiasma loops. Figs. 2*a*, 3*a*, 4*a* and 5*a* are sketches of the bivalents shown in Figs. 2, 3, 4 and 5 respectively. Chromosome type: 2, C; 3, E: 4, E; 5, B. K indicates the kinetochore and x the chiasma. From permanent smear preparations pretreated with water. ×3750.

which originated when the spiral was forcibly bent by the existence of the chiasma.

The direction of coiling in these loops was recorded using the symbols r and l for each turn, dextrorse and sinistrorse respectively. In recording it was provided that recording starts from the proximal chiasma or the kinetochore and ends at the distal chiasma. Thus for example the configuration represented in Fig. 6 was expressed as: $\frac{\text{r r r r r r r r r r r}}{\text{l l l l l lr r r r r r}}$, the numerator and denominator representing each of the two arms of the loop. The data obtained from such an analysis are tabulated in Tables 1, 2 and 3, for the true terminal, the apparent terminal and the interstitial types respectively.

Table 1. Structure of true terminal loops

No. of turns	Configurations	No. of reversals	No. of turns	Configurations	No. of reversals
3 (Cs)	$\frac{\text{l l l}}{\text{r r r}}$	$\frac{0}{0}$	6 (Bl)	$\frac{\text{r r l l l l}}{\text{l l r r r r}}$	$\frac{1}{1}$
4 (A)	$\frac{\text{l l l l}}{\text{r r r r}}$	$\frac{0}{0}$	7 (A) Fig. 8	$\frac{\text{l l l l l l l}}{\text{r r r r r r r}}$	$\frac{0}{0}$
4 (Bs)	$\frac{\text{l l l l}}{\text{r r r r}}$	$\frac{0}{0}$	7 (Cl)	$\frac{\text{l l l l l l l}}{\text{r r r r r r r}}$	$\frac{0}{0}$
4 (El)	$\frac{\text{l l l l}}{\text{r r r r}}$	$\frac{0}{0}$	7 (Dl)	$\frac{\text{r r r r r l l}}{\text{r r l l l l l}}$	$\frac{1}{1}$
6 (A) Fig. 7	$\frac{\text{l l l l l l}}{\text{r r r r r r}}$	$\frac{0}{0}$			

Table 2. Structure of apparent terminal loops
(Cases marked with asterisks are those obtained from X-rayed material, p. 417)

No. of turns	Configurations	Proportion of r to l	No. of reversals	No. of turns	Configurations	Proportion of r to l	No. of reversals
5 (Bl)	$\frac{\text{l l l l lr}}{\text{r r r l l}}$	$\frac{0.5:4.5}{3:2}$	$\frac{1}{1}$	6 (Bl)	$\frac{\text{r r r l l l}}{\text{r r r r r r}}$	$\frac{3:3}{6:0}$	$\frac{1}{0}$
5 (Cl)	$\frac{\text{r r r r r}}{\text{r r rl l l}}$	$\frac{5:0}{2.5:2.5}$	$\frac{0}{1}$	6 (Dl)	$\frac{\text{rl r r r r r}}{\text{r r r r r l}}$	$\frac{5.5:0.5}{5:1}$	$\frac{2}{1}$
5 (El)	$\frac{\text{lr r l l r}}{\text{r r r l l}}$	$\frac{2.5:2.5}{3:2}$	$\frac{3}{1}$	6 (A)	$\frac{\text{r r r rl l r}}{\text{l l r r r r}}$	$\frac{4.5:1.5}{4:2}$	$\frac{2}{1}$
6 (Bl)	$\frac{\text{r l r r r r}}{\text{r rl l l l l}}$	$\frac{5:1}{1.5:4.5}$	$\frac{2}{1}$	6 (A)	$\frac{\text{l l lr r r r}}{\text{r r r r r l}}$	$\frac{3.5:2.5}{5:1}$	$\frac{1}{1}$
6 (Dl)	$\frac{\text{r r l l l r}}{\text{lr r r l lr rl}}$	$\frac{3:3}{3.5:2.5}$	$\frac{2}{4}$	6 (B) Fig. 9	$\frac{\text{l l l l l l}}{\text{rl l l l l r}}$	$\frac{0:6}{1.5:4.5}$	$\frac{0}{2}$

Table 2. (*Continued*)

No. of turns	Configurations	Proportion of r to l	No. of reversals	No. of turns	Configurations	Proportion of r to l	No. of reversals
6 (Bl)	l l l l l l r r r l l l	0 : 6 3 : 3	0 1	7 (Bl)	l lr r r r r r r r r l l l l	5.5 : 1.5 3 : 4	1 1
7 (A)	l l lr r r r r l l l lr r r r	4.5 : 2.5 3.5 : 3.5	1 1	8 (Cl)*	l l l l r r r r l l l l l l l l	4 : 4 0 : 8	1 0
7 (A)	l l l lr r rl r l l r r r r r	3 : 4 5 : 2	3 1	8 (A)	r r r r r rl l l r rl l l l l l l	5.5 : 2.5 1.5 : 6.5	1 1
7 (A)	l l r r r r r r r lr l l l r	5 : 2 3.5 : 3.5	1 4	10 (A)	r r r r r r r r r r l l l l l lr r r r r	10 : 0 4.5 : 5.5	0 1
7 (Bl)	r r r r l l l l lr r r r r r	4 : 3 5.5 : 1.5	1 1	11 (A)* Fig. 6	r r r r r r r r r r r l l l l l lr r r r r r	11 : 0 5.5 : 5.5	0 1

Table 3. Structure of interstitial loops

No. of turns	Configurations	Proportion of r to l	No. of reversals	No. of turns	Configurations	Proportion of r to l	No. of reversals
3 (A)	r r r l l l	3 : 0 0 : 3	0 0	4 (Cl)	r r r r lr r r l	4 : 0 2.5 : 1.5	0 2
4 (Cl)	r r l l r r l l	2 : 2 2 : 2	1 1	4 (Cl)	l l l l l l l l	0 : 4 0 : 4	0 0
4 (Cl)	r r l l l l l l	2 : 2 0 : 4	1 0	4 (Bl)	r r r r l r r r	4 : 0 3 : 1	0 1
4 (Dl)	r r r r l l l lr	4 : 0 0.5 : 3.5	0 1	5 (A)	l r l r rl r r r rl r	2.5 : 2.5 4.5 : 0.5	4 2
4 (Bl)	l l r r l r r r	2 : 2 3 : 1	1 1	5 (Bl)	r rl l l r l rl l r r	2 5 : 2.5 2.5 : 2.5	2 3
4 (El)	r l l r r r rl l	2 : 2 2.5 : 1.5	2 1	5 (Cl)	r r r r r r r r r r	5 : 0 5 : 0	0 0
4 (Dl)	r r r rl l l l l	3.5 : 0.5 0 : 4	1 0	5 (Cl)	l lr r rl l r r r r r	2 : 3 5 : 0	2 0
4 (A)	r rl l lr l l l l	2 : 2 0 : 4	2 0	5 (Cl)	l l l l l l l r r r	0 : 5 3 : 2	0 1
4 (Dl)	r r r r l l l l	4 : 0 0 : 4	0 0	5 (Dl)	l l l r r r r r r rl	2 : 3 4.5 : 0.5	1 1
4 (Dl)	r r rl l l l l	3.5 : 0.5 0 : 4	1 0	5 (Dl)	l l l r r r r r rl l	2 : 3 3.5 : 1.5	1 1
4 (A)	r r r l l l l l	3 : 1 0 : 4	1 0	6 (A)	r r r r r r r l l l l l	6 : 0 1 : 5	0 1
4 (A)	r r r r l l l l	4 : 0 0 : 4	0 0	6 (A)	r r r r r r r r rl l l r	6 : 0 3.5 : 2.5	0 2

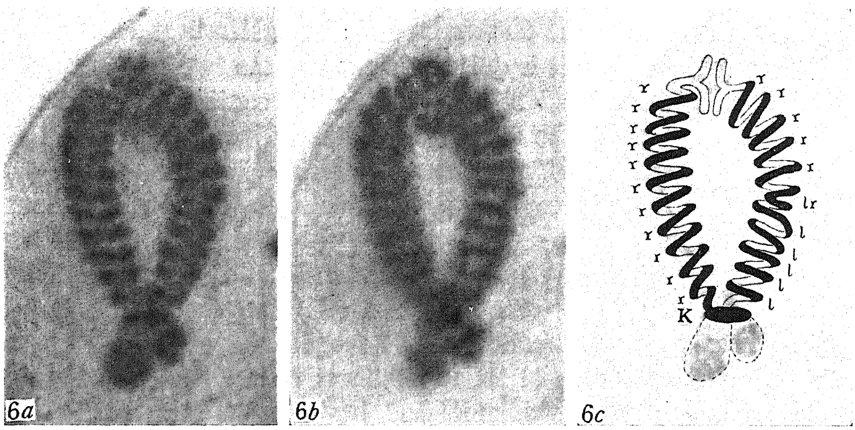
Table 3. (*Continued*)

No. of turns	Configurations	Proportion of r to l	No. of reversals	No. of turns	Configurations	Proportion of r to l	No. of reversals
6 (A)	l lr r rl l l	2 : 4	2	7 (A)	l l l l l l l	0 : 7	0
	l l l l l r	1 : 5	1		l lr r r r l l	3.5 : 3.5	2
6 (A)	l l lr rl lr r	2.5 : 3.5	3	7 (A)	r r r rl l r r	5.5 : 1.5	2
	l l l r r r	3 : 3	1		r r rl l l l l	2.5 : 4.5	1
6 (Cl)	l l l l l l	0 : 6	0	7 (Cl)	r r r rl l l l	3.5 : 3.5	1
	l l l r rl l	1.5 : 4.5	2		r r r r rl l l	4.5 : 2.5	1
6 (Cl)	l l l l l l	0 : 6	0	7 (Dl)	l l l l l l l	0 : 7	0
	r r r r l l	4 : 2	1		r lr r r rl l l	4 : 3	3
6 (Cl)	l l r rl l l	1.5 : 4.5	2	7 (Dl)	l r r r r r r	6 : 1	1
	l l l l l l	0 : 6	0		r r r r r r r	7 : 0	0
6 (Cl)	l l l l l l	0 : 6	0	7 (Dl)	r rl lr r r r r	6 : 1	2
	r r r r r r	6 : 0	0		l l r r rl l l	2.5 : 4.5	2
6 (Dl)	r r l l l r	3 : 3	2	9 (Dl)	l l l l l r l r l	2 : 7	4
	r r l l l r	3 : 3	2		r r r r r r r l l	7 : 2	1
6 (Dl)	l l l l l l	0 : 6	0				
	r r r r r r	6 : 0	0				

Table 4. Summary of Tables 2 and 3
(In the second column "i" denotes interstitial loops and "t" apparent terminal ones)

No. of turns	No. of loop-arms observed		Frequency of the balanced type (its percentage)	Frequency of reversals (per 100 turns)
3	i	2	0 %	0 %
4	i	28	6 (21.4)	17 (15.2)
5	t i	6 14 } 20	2 3 } 5 (25.0)	7 18 } 25 (25.0)
6	t i	16 20 } 36	3 3 } 6 (16.7)	21 19 } 40 (18.5)
7	t i	10 12 } 22	2 2 } 4 (18.2)	15 15 } 30 (19.5)
8	t	4	1	3
9	i	2	0	5
10	t	2	0	1
11	t	2	1	1

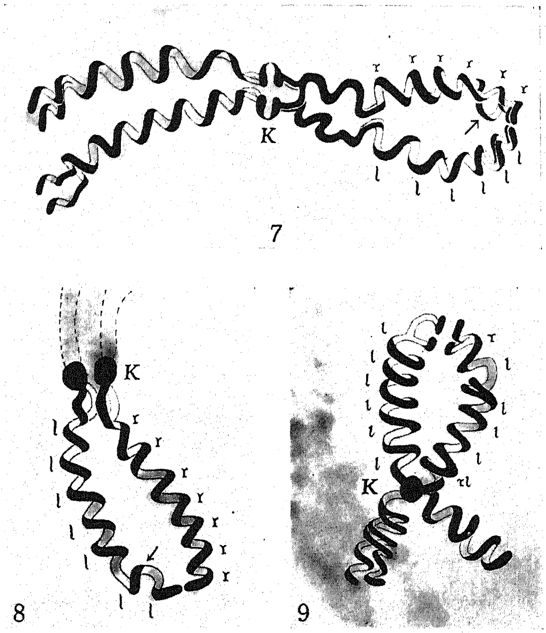
From Table 1 it will be inferred that in the true terminal loop the component two arms behave as if they were a single continuous thread. Among the nine cases analyzed, seven were of the type in which one loop-arm coils in one direction and the corresponding arm in the reverse direction, the terminal chiasma being the point



Figs. 6. Representing the direction of coiling in a large chiasma loop of a bivalent C. The photos were taken in two different foci (*a* and *b*) from an aceto-carmine preparation pretreated with water; it came from X-rayed material, though no apparent deformity exists in this bivalent constitution, excepting that the number of turns is much larger than that in the control. Note that in the right arm of the loop the reversal of coiling direction has occurred exactly at its middle point. K, the kinetochore; *l* and *r*, sinistrorse and dextrorse turns respectively. ×ca. 3000.

of the reversal (Figs. 7, 8). In the other two cases a reversal occurred once in each arm, but in such a way that the total number of dextrorse turns is exactly equal to that of sinistrorse turns. These findings suggest that the spiral assumed is of balanced type, although it was not possible in the present study to verify this by the internal spatial relationship of the paired chromatids in these loops at the time when they take the form of fused spirals.

On the contrary in cases of apparent terminal and inter-



Figs. 7–9. Coiling direction in chiasma loops. These figures are reproduced from Figs, 4, 2 and 3e, respectively, in the previous paper, No. 14, Chromosome type: 7 and 8, A; 9, B. The arrows in Figs. 7 and 8 indicate distortion in the pitch of the spiral.

stitial chiasma loops the data presented in Tables 2 and 3 show no such regularity. Here it is quite evident that the two arms of a loop are subjected to independent spiralisation, the continuity of the arms being interrupted by the interstitial chiasma. In general there is no clear demarcation between these two kinds of chiasma loops with respect to the spiral configurations assumed; both of them are likewise characterized by (i) very frequent occurrence of reversals in coiling direction, (ii) no correspondence in coiling direction in the corresponding arms of a loop and (iii) frequent occurrence of the balanced type of spirals. Actual frequencies of the occurrence of the balanced type and of the reversals in coiling direction in these two kinds of loops are summarized in Table 4. From the data presented in these tables the above three features may be statistically grounded as follows.

(i) The frequency of loop-arms with one or more reversals was 81 among the total 118 arms observed, that is, 68.6%. Such high percentage makes a marked contrast with the value, 34.0%, previously found in chromosome arms whose distal ends are free (No. 4 of this series). Moreover one meets here frequently with cases of double, triple or even quadruple reversals occurring within a single loop-arm, a condition entirely incomparable with cases previously studied on free arms in which among the total 986 arms under observation only two **Dl** arms (the longest arm of a complement) were found to show the maximum triple reversal. In free arms the frequency of reversals is directly proportional to the length of arms, whereas in the case of chiasma loops such a relation seems not to exist (cf. Table 4).

(ii) The balanced type of spiral was found in 24 arms among 118, that is, 20.3%. Such high frequency can not be attributed to mere chance. Furthermore consideration must be paid to the frequent occurrence of forms which are "nearly balanced", such as expressed by proportions of the opposing turns as 2:3, 3.5:4.5 etc. It may be noticed also that the loop length probably does not bear any intimate connection with the occurrence of the balanced type (cf. Table 4).

(iii) That there is no correspondence in the configuration between the corresponding arms of a loop is well illustrated by Fig. 6. In this bivalent one arm is of the balanced type, whereas the other arm is of the unbalanced type throughout its spirals. A statistical basis for this conclusion will be given as follows. Let us consider only the direction in the first proximal and the last distal coils of these loops. The three kinds of configurations in the proximal coil,

$\frac{r}{r}$, $\frac{l}{r}$ and $\frac{l}{l}$, will freely combine with the same kinds of configurations in the distal part. The resultant nine classes of configurations and actual frequencies in each are given in the following (t = apparent terminal, i = interstitial):

	$\frac{r}{r}\cdot\frac{r}{r}$	$\frac{r}{r}\cdot\frac{l}{r}$	$\frac{r}{r}\cdot\frac{l}{l}$	$\frac{l}{r}\cdot\frac{r}{r}$	$\frac{l}{r}\cdot\frac{l}{r}$	$\frac{l}{r}\cdot\frac{l}{l}$	$\frac{l}{l}\cdot\frac{r}{r}$	$\frac{l}{l}\cdot\frac{l}{r}$	$\frac{l}{l}\cdot\frac{l}{l}$
t	0	4	1	4	7	1	2	1	0
i	3	3	2	3	13	7	2	2	4
Total	3	7	3	7	20	8	4	3	4

The results are close to the expected ratio of 1:2:1:2:4:2:1:2:1, respectively, though a certain excess exists in the $\frac{l}{r}\cdot\frac{l}{r}$ class. With respect to the proximal coil, the three types, $\frac{r}{r}$, $\frac{l}{r}$ and $\frac{l}{l}$, occur in 13, 35 and 11 arms respectively, and likewise the corresponding types of the distal coil occur in 14, 30 and 15 arms respectively. In both cases the data are accordant with the expected 1:2:1 ratio.

ii) Bridges

The present study on the structure of bridges is based on material irradiated on December 12, 1939. The X-ray apparatus used was the Shibuya Co's SP2 and the treatments were: 60 KV, 15–20 ma, 20 cm unscreened, for two minutes. At the time of treatment the PMCs were at resting stage; for about two weeks after irradiation the material was kept in a hot chamber of 20°C till they attained the first anaphase or telophase. The PMCs were smeared according to the writer's pretreatment method (No. 11 of this series) and fixed and stained with aceto-carmine. Twenty-five chromatid bridges in 20 cells at first anaphase or telophase were recorded with respect to the direction of coiling of the chromonema. These are tabulated in Table 5; some of them are illustrated in Figs. 10–13. Some of these data are however incomplete owing to difficulties often encountered in recording the chromonema coiling at the end regions of bridges where exact observation was prevented by clumping of other chromosomes.

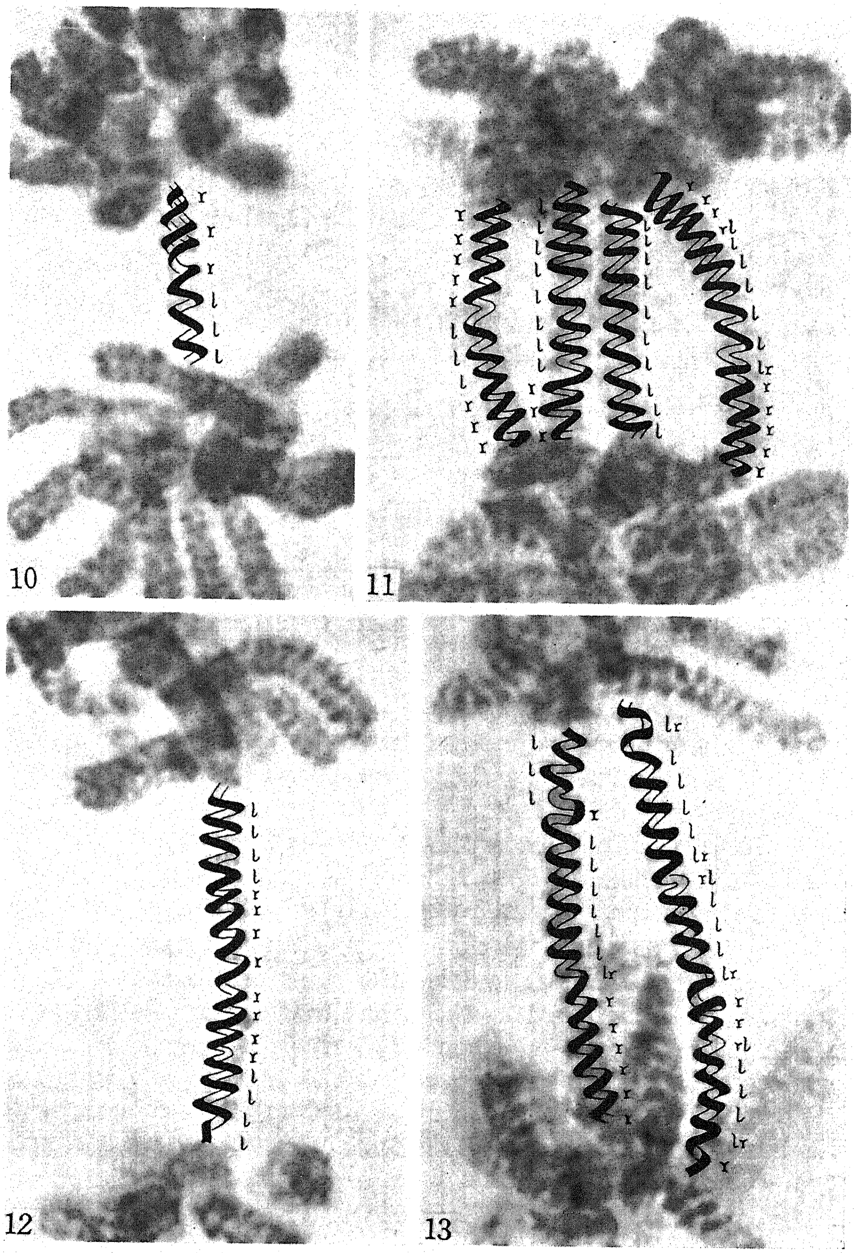
The mode of formation of chromatid bridges in meiosis is various; they originate in both intra-chromosomal and inter-chromosomal ways (No. 9 of this series). The most simple case is the intra-arm bridge, that is, a bridge resulting from break and fusion at the corresponding loci of the paired chromatids within an arm of a bivalent. The spiral configurations to be assumed by such bridges

Table 5. Structure of chromatid bridges

Cell No's	Configurations	Proportion of r to l
1	r r rl l l	2.5 : 2.5
2 Fig. 10	r r r l l l	3 : 3
3	l l r r r rl r	4.5 : 2.5
4	l r r l r l l	3 : 4
5	l l l l l l l	0 : 7
6	l l l l l r r r	3 : 5
7	l l l l l l l l	0 : 8
8	r r r l l l l l l r	4 : 6
9	r r r l r r r r l l l	7 : 4
10	l l l l r r r r l r r	6 : 5
11 Fig. 11	r r r r r l l l r r r	8 : 3
11 ,, ,,	l l l l l l l l r r r	3 : 8
11 ,, ,,	l l l l l l l l l l	0 : 10
11 ,, ,,	r r r rl l l l l l lr r r r r r	9 : 6
12	l l l r r r r r r r r r	9 : 3
12	l l l l l l l l l r r r l r	4 : 9
13	l l r r r r rl l l lr r r r	8 : 5
14	l l l l l lr r r r r r r r	7.5 : 5.5
15	r r r rl l l l l l r rl l l l l	5 : 10
16	l l l r r r r rl l l l l l lr r	6 : 9
17	r r r l l r r r r l l l l r r	9 : 6
18 Fig. 12	l l l l r r r r r r r r l l l l	8 : 8
19	r r l l l l l l l l l l l r l l l l l	3 : 15
20 Fig. 13	l l l r l l l l l l lr r r r r r r	7.5 : 9.5
20 ,, ,,	lr l l l l lr rl l l l lr r r rl l l l lr r	6 : 13

are expected not to be essentially different from those in free arms, since the fused ends of the broken chromatids of an arm would permit free rotation in spiralisation. On the contrary the bridges which came from the chromatid fusion between different arms of a bivalent or of different bivalents may show very complicated configurations, because here in addition to the fixed points at the ends (the kinetochores) there is further inserted another point of fixation at the fused place. A thread with such three fixed points will provide configurations more complicated than a thread does whose ends only are fixed. Thus in the case of chromatid bridges the spiral configurations are expected to range from very simple to very complicated ones. The data presented in Table 5 are taken as generally suggesting such variable results. The bridges illustrated in Fig. 11 are rather "smooth", while the bridge on the right side in Fig. 13 is very "rugged".

It was sometimes possible to discern half-chromatids in some parts of bridge spirals (Fig. 10), but no case was met with in the present preparations in which their identification was possible throughout the entire length. Owing to this disadvantage, it was



Figs. 10–13. Representing the structure of chromatid bridges in anaphase of irradiated PMCs. Aceto-carmine smears pretreated with water. ×ca. 2600.

not possible to gain an insight into the internal structure of bridge spirals. Such a configuration as llllrrrrrrrllll (Fig. 12) might be of the balanced nature, one of the broken chromatids being llllrrrr of the balanced type and the other rrrrllll of the same nature and the fusion having occurred at the two r ends of these. This interpretation is not however conclusive, for there are possibilities of other interpretations.

Owing to these additional variables, the present study on bridges is merely of a significance subsidiary to the conclusions drawn from the observations on chiasma loops.

By the way it may be noted that the fused ends of chromatids never retain any trace of the fusion. It is certain that every bridge should have at least one locus of fusion; nevertheless, it is not possible to identify these loci in such bridge spirals as illustrated in Figs. 10–13, the reunited chromonemata being morphologically entirely indistinguishable from the unbroken ones. Such will be taken as one of the most characterizing features of the chromonema.

Discussion

As stated in the Introduction, the following two subjects constitute the principal items of the present discussion:

1) Are the paired chromatids already twisted about each other at the time of opening-out?

2) How can the chromonemata be spirallized?

The first question is directly related with the second one, because if it be admitted that the paired chromatids are in a twisted state at the initiation of diplotene separation, these twists would bear a certain relation to the spirals which are to be later assumed, whereas if the paired chromatids lie parallel at this stage, the problem would be entirely different. The first question then must be considered first.

Kuwada ('39) believes that synapsis between the homologues takes place while they are undoing the twists which came from the relational spirals assumed by the paired half-chromatids at the last premeiotic anaphases, and when such untwisting occurs in certain parts of the two homologous threads which happened to be twisted in the same direction, intertwining and subsequently chiasmata will be formed between them in such a manner as suggested by Darlington ('35).

In opposition to this view, the writer's Neo-two-plane theory of bivalent constitution is based on the assumption of free opening-out, two-by-two, of the four strands. This condition can be effected only

if the four strands lie essentially parallel. That actually both modes of opening-out of the pair along the synaptic and the equational planes occur in the expected 1:2 ratio has been statistically demonstrated by the writer in two lines of observations (No. 7 of this series). It may be added further that in "precocious bivalents" each of the paired chromatids is independently subjected to spiralisation and lies parallel without any sign of interlacing about each other from the very beginning of diplotene up to metaphase (see Figs. 3–7 Plate 2, No. 5 of this series).

Now the data presented in this paper are taken as favoring the writer's view. In contradistinction to the findings in cases of free arms previously studied (Nos. 2 and 4 of this series), in cases where both the ends of the paired chromatids or of their segments are mechanically fixed in spiralisation, the configurations assumed by these loops were found to be characterized by (i) incapability of assuming regular cylindrical spirals when the loops are very short,[1] (ii) occurrence, though rare, of independent coiling in each of the paired chromatids, (iii) profound increase in frequency of reversals in coiling direction, and (iv) frequent occurrence of the balanced type of spirals (cf. Fig. 1a). These characteristic configurations shown by loops can not be ascribed to any internal preexisting difference of them from free arms but merely to the differrence that in free arms their distal ends are literally free in the process of spiralisation, while in loops the two ends are fixed and can not rotate at all so that a considerable stress is thereby imposed upon them at the time when they are subjected to spiralisation. If Kuwada's concept of spiral formation be correct, the twists supposed as preexisting between the paired chromatids would not be able in the case of loops to release at all and would remain intact, while in free arms they could be untwisted as the chromosomes contract and increase in diameter, and so the consequence would be the formation of relational spirals to a much higher extent in loop-

1) It may be noted here that in spite of this fact the chromosomes at anaphase (i. e., half-bivalents) show no longer any sign of such peculiarities. This must imply that wavy configurations assumed by such loops can be easily transformed into regular spirals as soon as the impediment set up by the chiasma was removed at late metaphase where the paired chromatids completely separate from each other. It is supposed then that this conversion from zigzag to spiral configurations will occur independently in each of the paired chromatids of such loops, and consequently different spiral configurations may be sometimes assumed by each of them. This, together with the fact described under item (ii) in the text, is regarded as responsible for the exceptional cases with which the writer met in his analytical study on the configurations in half-bivalents (No. 3 of this series, p. 166), that is, for the configurations which "can not be understood unless one assumes the occurrence of independent coiling of chromatids in a certain segment."

arms than in free ones. Such a conclusion is actually at variance with the present findings. Especially the fact that in the case of terminal chiasma loops the spirals are always of the balanced type invalidates the assumption of twists remaining at the time of chromatid opening-out.

It is now clear that in terminal chiasma loops the distal ends of the chromatids are connected so tightly that the two loop-arms behave in spiralisation as if they were a single continuous thread. Probably such intimate connection of the distal ends of the arms may be maintained by the union of the matrix at this region. Such a significance of the matrix has been pointed out by Huskins & Smith ('34), Sax & Humphrey ('34) and Sax ('35). In these terminal chiasma loops, the component arm always has a mate which is exactly the same in length and therefore the two arms of each loop are in such a relation that the stress originated in one arm by spiralisation can be completely cancelled out by the other arm. On the contrary, in interstitial chiasma loops (including apparent terminal ones) each of the two arms of a loop is subjected independently to spiralisation, as it has no such a mate to be compensated, and hence it must readjust in itself for the spiralisation factors (vide infra). Probably such difference in condition between terminal loops and interstitial ones may be the reason why in the former the configurations are always of the balanced type, while in the latter they are not always of the same type.

The inference that the four strands lie essentially parallel at the initiation of chromatid opening-out will naturally lead to an assumption that synapsis implies parallel conjugation between the homologues which are free from internal twisting. The same idea was expressed by Cooper ('38) as follows: "...... at meiosis, the synapsing chromosomes are bilateral in organisation, i. e., constructed in such a manner that each possesses but one, limited, pairing surface". Friedrich-Freska ('40) has interpreted such a bilateral organisation of chromosome threads in electrostatic terms. Then it may not be amiss to say further that the essential condition prerequisite to chromosome synapsis is the untwisting of relic twists of the homologues. Such relic twists in meiotic prophase are certainly as stated by Kuwada ('39) the remnant of twists which originated from the relational spirals of the two half-chromatids within each chromatid at the premeiotic division. In this connection the facts that leptotene threads are, as well known, extensively elongated (cf. Nebel and Ruttle '37) and that the meiotic prophase is usually characterized by considerable lengthening of its duration (cf. Beasley '38) will be regarded as supporting the present view. It

was shown by the writer (Nos. 5 and 8 of this series) that by speeding up the division by high temperature meiosis can be converted into mitosis, pairing being entirely prevented. On the other hand, in mitosis the relatively rapid prophase and hence incomplete untwisting of homologues will prevent pairing between them. In fact the mitotic chromosomes are known usually to maintain relic twists still up to the metaphase, though the decrease in their number gradually occurs from prophase to metaphase (cf. Husted '38).

To conclude, the answer to the first question is thus:—At the time of chromosome pairing each homologue is in the untwisted condition, pairing takes place then along the given surface of the synapsing chromosomes, and hence after opening-out the paired chromatids lie parallel to each other.

Now the second question which is concerned with the cause of the coiling of chromonemata should be then considered on the basis of the above conclusion. Students in this field, such as Kuwada ('27), Sax ('30), Sax and Humphrey ('34), Darlington ('35), Huskins and Smith ('35), Nebel ('39), etc., have all believed that the two chromatids of each chromosome should coil in such a manner that for each turn of the spiral there is a twist of the two chromatids about each other in the reverse direction, that is, that the two chromatids assume the parallel spiral system of the writer's terminology, so they can freely separate from each other without entangling, and that such a spiral can be produced without rotating or twisting of the chromatid ends about each other. Such an argument is based on nothing but teleological grounds, without ascertaining by direct observations such a spatial relationship of the paired chromatids within each chromosome. Contrary to the expectation of these workers the fact was revealed by the writer (No. 12 of this series) that at early metaphase the paired chromatids constitute the relational spiral system, so that when the spiral is drawn out without rotating the ends, they are twisted about each other in the same direction and in the same number of twists as the spiral turns.[1)]

Since, as inferred above, the paired chromatids lay parallel to each other at the time of opening-out, such a twisted orientation in space of the coiled chromatids must be regarded as having originated during the process of spiralisation. If such relational spirals were

1) Kuwada ('38) observed likewise such an arrangement of the paired chromatids in the first meiocyte of *Tradescantia*. He considers however such a situation as representing "a special case" in contrast with "the ordinary case where the two chromatids are freely separable from each other" ('39, p. 248).

always of the balanced type as in the case of plant tendrils, it will be inferred that the chromosome ends were fixed in spiralisation. The configurations actually observed however do not fit this assumption. On the contrary the fact of the existence of spirals relational and without any reversal in direction throughout the entire length of an arm and furthermore the fact that the configurations in loop arms greatly differ from those in free arms strongly suggest that in free arms the free ends are literally free, being able to rotate in spiralisation. Of course the writer does not intend to insist that there is no resistance at all to the entirely free rotation of the paired chromatids, but it may be more reasonable to assume that the viscosity of the matrix in which the chromonemata are inbedded counteracts it to a certain extent. Probably such a resistance may be the cause of reversals in coiling direction.

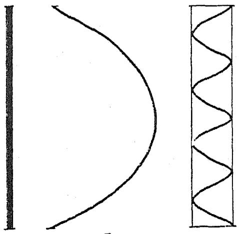
Next a question is to be raised concerning what factors are operative for chromonema coiling. The first to be considered is the matrix contraction, as already considered by nearly all investigators referred to above excluding Darlington. The chromonemata are certainly elastic and flexible to a certain extent, and hence as the surrounding matrical substance contracts the paired chromatids will be forced to take a zigzag wavy configuration within this limited space. The paired chromatids behave here as if they were a single thread, the association between them being so close. Thus the resultant configuration is comparable with that to be formed by a flexible wire when it was compressed in a glass tube. Owing to the elastic property of the chromonema, the corrugated string is supposed to maintain its untwisted orientation in space. Probably such zigzag configurations are such as those actually observed in prophase chromosomes.

The matrix contraction can however not be considered as the sole agent for chromonema coiling: it seems to be insufficient to cause such a regular spiral form as observed in metaphase chromosomes, that is, so uniform pitch and so striking regularity of the spiral (cf. No. 1 of this series). The contraction of the matrix is supposed to take place gradually from prophase to metaphase or anaphase, while the completion of the spiral does not appear to be gradual; the zigzag appearance of the chromonema seen until diakinesis suddenly changes into the regular spiral as soon as the chromosomes come to the equator at metaphase. Such an apparent sudden changes in configuration should be ascribed to a factor or factors connected with the characteristic nature, plasmic or chromosomal, of the cell at metaphase. The writer interpretes, though tentatively, this change as follows.

It seems likely that the repulsion and attraction which act upon chromosomes are electro-magnetic factors, as evidenced by the observations that the arrangement of individual chromosomes on a metaphase plate is similar to that of floating magnets in an electro-magnetic field (cf. Kuwada '29). From the cataphoresis experiments (cf. Wilson '25) and the staining reactions (Kuwada and Sugimoto '28) it has been suggested that the chromosomes which were negatively charged before the disappearance of the nuclear membrance change their charges when they come to the equator probably owing to the presence of a strongly positive-charged portion of the cytoplasm at this region. It will be reasonable to assume then that such a sudden change in the charge of the chromosomes is co-operative to the sudden change in their internal structure. It is supposed that the positive charges now accepted by the chromosomes are so strong that the components of the chromonema itself are strongly repelled from each other. This would cause the string to elongate as much as the matrical space can permit, and the consequence will be a regular spiral, the pitch of which can be determined by the equilibrium between the strength of such internal repulsion force, the elasticity of the chromonema and the external limitation of the matrix. In the transformation of the zigzag arrangement to the regular cylindrical spiral, the free end of the string will rotate to readjust to this conversion and hence the coiled string is supposed to maintain its untwisted orientation. In chiasma or bridge loops both the ends are fixed and therefore a certain difficulty will be imposed upon the paired chromatids to readjust regularly; in these the most suitable readjustment may be the formation of spirals of the balanced type, as demonstrated in the present study. In small loops however the situation is quite different. Here the two strings (a pair of paired chromatids) are embedded in a common matrix, the separation of the matrix being prevented by the loops. These two strings will tend to lie, as far as possible owing to their mutual repulsion, within the limiting space of the matrix which is necessarily flat in dimensions. This mutual repulsion of the two strings will be counteracted by their elastic property, so that they will tend to have contracting points as few in number as possible. Such a condition will naturally lead to the formation of wavy and symmetrical configurations in these loops, as shown in Figs. 2–4 of the present paper.

It was sometimes argued which of the major and the minor spiral originates first in meiosis. Both Sax ('36) and Darlington ('37) consider that the minor spiral is formed first, while Kuwada and Nakamura ('33, '34) maintain that the major spiral comes

first. Based on the above postulations on the major spiral, the writer is inclined to assume that the minor spiral is the consequence of extensive elongation of the string which is brought about at metaphase by the development of strong repulsion force between each component of the string itself. In prophase chromosomes the string will be in a contracted state, as it is compressed by the matrix contraction (Fig. 14 a). When the repulsion force develops internally, the string will elongate (Fig. 14 b). However if this elongation of the string is restricted to take place within certain limits of space, the result will be the formation of regular spirals Fig. 14 c). Such a limitation of space may be ascribed to the development of the minor matrix (cf. No .12 of this series) which is supposed to have differentiated from the surrounding major matrix during the process of spiralisation.



a *b* *c*

Fig. 14. Diagrams showing the mode of development of the minor spiral. *a*, a contracted string; *b*, the same string elongated in unlimited space; *c*, the development of minor coils as the result of elongation within a certain limited space, as within a glass tube.

In short **the metaphase** (or anaphase) **chromosomes are regarded as the maximum contracted form externally, but the maximum expanded form internally.** The equilibrium between the external and the internal agencies is regarded as responsible for the formation of regular spirals, both major and minor.

Summary

In the present paper the configuration assumed by the chromonema in chiasma loops and chromatid bridges was studied, with an aim to ascertain how the chromonema coiling in such arms both ends of which are fixed differs from the previous findings on free arms whose distal end is free and to touch in this way of comparison the problem of the mechanism of spiralisation. Observations on these "fixed" strands have shown that:—

1) When they are below a certain limit of length, they are not able to assume regular cylindrical spirals, but take merely wavy corrugated configurations. In "free" strands no such a conditions is met with.

2) Sometimes each of the paired chromatids is subjected to independent coiling. In free arms of the normal chromosomes such irregularity in coiling is never met with, the paired chromatids always forming a single fused spiral.

3) The spiral configurations assumed by these, when they are relatively long, are characterized by a profound increase in frequency of reversals in coiling direction as contrasted with the case of free strands previously studied.

4) In these strands the spiral of the balanced type (Fig. 1a) occurs predominantly; especially in the true terminal chiasma loops, the configurations are always of this type.

From these findings and other sources of evidence previously presented it has been inferred that:—

1) Pairing of the homologues takes place after the leptotene threads completed the untwisting of the relic twists; they conjugate thereafter side-by-side in one, limited pairing surface of each; the four strands which are to separate two-by-two at diplotene lie thus essentially parallel.

2) "Free" strands are able to rotate in spiralisation and consequently the parallel arrangement of the paired chromatids can be converted into the twisted orientation in spirals, that is, the relational spiral, while in "fixed" strands their freedom of rotation is prevented and therefore the paired chromatids take more complicated configurations as the present paper has shown. In the latter, the paired strands will assume either a relational spiral in the form of a balanced spiral or a parallel one in the form of an unbalanced spiral.

3) For what determines the pitch and the regularity of the spiral in metaphase chromosomes the following three factors are considerable: (a) the space delimited by the matrix, (b) the elasticity of the chromonema and (c) the electrical charges to be accepted by the chromonema at metaphase plate which determine at the same time the external chromosome mechanics. These are responsible for the formation of the major as well as the minor spiral.

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Studies of Mitosis and Meiosis in Comparison
II. Chromosome structure in the spiral stage and anaphase in mitosis as revealed by means of a maceration method

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While the iron-acetocarmine smear method has been widely used in the study of the internal structure of the meiotic chromosomes, this method is not generally found advantageous in the study of the somatic chromosomes, chiefly owing to the fact that in the latter case it is necessary to cut the tissue in section for the microscopic investigation. The "Kochmethode" of HEITZ (1926) and his improved "Nukleal-Quetschmethode" (1936) may serve for the purpose of this study of somatic chromosomes without cutting the material, but in the present investigation both these methods were found unsatisfactory for a closer investigation of the chromosome structure. After a number of attempts to obtain a suitable method, a maceration method was found successful in the cases of the root-tips of *Tradescantia reflexa*, *Vicia faba* and *Secale cereale*. In the present paper the results obtained with this maceration method in the root-tips of *Tradescantia reflexa* are reported. They contain two important facts in comparing mitosis with meiosis, concerning the chromosome structure at the spiral stage which seems to be different from that of the same stage in meiosis and the chromosome structure at anaphase which corresponds with the "tertiary split" in meiosis and also explains the origin of the major spirals in the same (comp. KUWADA, 1939).

Before going further, I wish to acknowledge my gratitude to Prof. Y. KUWADA under whose direction this investigation was carried out.

Method

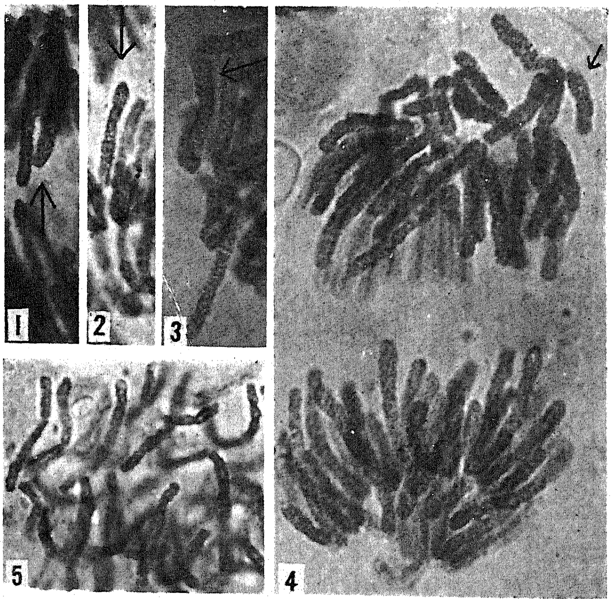
The root-tips of *Tradescantia reflexa* in spring when the plant is sprouting were used as material.

Root-tips cut off in suitable length from the plant were immersed in acetocarmine (45% acetic) or 45% glacial acetic acid for 10–30 minutes and then transferred to a mixture of 2 parts of conc. HCl and 1 part of 94% alcohol for 5–10 minutes. They were rinsed

in 45% glacial acetic acid 3 times, and finally immersed in aceto-carmine solution for 20–60 minutes. Each root-tip thus treated was then placed on a slide with a drop of acetocarmine, and a pressure was given on the cover glass to flatten the tissue. In the present investigation in most cases the root-tips were treated before the maceration, with a solution containing a few drops of commercial ammonia water in 100 c.c. distilled water for 5–7 minutes. As a result of this pretreatment with ammonia, it was possible to reveal the fine structure of the chromosome more clearly. When the influence of ammonia was too strong, the natural distribution of the chromonemata was of course affected, the effect being stronger at the outer or peripheral part of the root-tip and weaker in the inner or central part.

Observation

Anaphase. In Figs. 1 and 2, in the chromosomes indicated by arrows, two chromonema spirals (chromatids or half-chromosomes) clearly separated from each other are shown. Such figures of anaphasic chromosomes with two spirals clearly separated are met with only very rarely[1]. Usually the two spirals are found in the chromosome arranged longitudinally in close contact with each other (Fig. 3), though even in these cases, twisted figures of the spirals are often clearly observed (Fig. 4, comp. Fig. 5*b* of KUWADA, 1939).



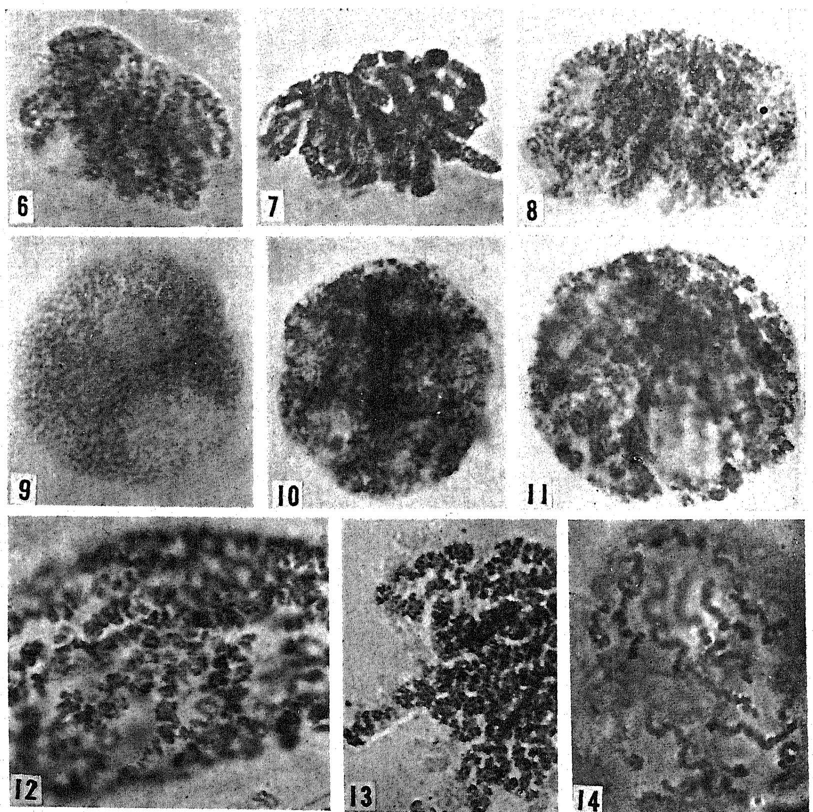
Figs. 1–5. Anaphase. Figs. 3 and 5 in early anaphase, and Figs. 1, 2 and 4 in later stage.

1) HUSKINS (1937) has also observed "the chromosome split (not only the chromonema double) in root-tip and microspore mitotic anaphases in a few rare cases." In both the preparations which we made with the ordinally paraffin method and with the maceration method not preceded by the ammonia pretreatment, the figures of the anaphase chromosomes showing two distinct spirals were not observed.

It is not in every case possible directly to observe that the anaphasic chromosome contains two spirals which are independent from each other, not forming a relational spiral or a form of the double-stranded spiral; but it seems highly probable from the configurations of the chromosomes, referred to above, showing twisting or a clear space between two spirals, that there are two spirals in the chromosome which are independent spirals rather than a double-stranded spiral. It is also a noticeable fact that each of the two spirals (chromatids), sometimes has the aspect of a double spiral as shown in Fig. 3.

On the structure of the somatic chromosome in the anaphase and telophase, the majority of authors seem to agree in the view that each chromosome consists of two threads more or less spirally interlaced with each other. But this cannot yet be called a generally accepted opinion. DARLINGTON (1937) denies the duality of the chromosomes. NEBEL (1933) has, on the other hand, asserted the quadripartite nature of the chromosomes in these stages describing it thus:— "four spirals running in four columns parallel to one another". GOODSPEED, UBER and AVERY (1935) have reached a similar conclusion in their study with ALTMANN's freezing-drying technique. NEBEL and RUTTLE (1936) have observed four chromonema threads per chromosome through all the mitotic stages except the metaphase, where, the authors consider, the threads are doubled, their actual multiplication being assumed as occurring at this or an earlier stage. MARSHAK (1936) has reported, in his study of somatic mitosis in several plants, that two chromonemata are found in each anaphase or telophase chromosome, and he considers that there are two types of coiling:—1) Each chromonema independently coils in H_1 ("the smallest visible helix"), and 2) the two such chromonemata are more or less spirally interlaced to form H_2 "of larger diameter than H_1". According to MARSHAK, the pair of H_1 apparently separate from one another in the telophase except for the spindle fibre attachment region. The results of the present investigation of anaphase chromosomes in *Tradescantia reflexa* conform with the view that each anaphase chromosome consists of two chromatids. The two chromatids sometimes reveal themselves to be more or less twisted around each other, and in each chromatid the minute spirals of MARSHAK are observed. In some of the chromosomes, the figure which indicates that each chromatid contains a double spiral is also observed (Figs. 1, 3, indicated by arrows), which is in harmony with the view of four threads per chromosome of NEBEL. In our preparations, however, the single chromonemata seem not quite separate from one another in anaphase and telophase (cf. NEBEL and RUTTLE, 1936).

Telophase and *interphase*. When the chromosomes reach the poles, they undergo the maximum contraction (Fig. 6). They soon begin to elongate again, however, and the coiled chromonemata come to expand (Figs. 7, 8). A nuclear membrane is organized and the nucleoli make their appearance; at first small, they gradually increase in size, some being united with one another. They show no staining affinity to acetocarmine, and each of them reveals itself as a hyaline mass of round shape, thus in the vicinity of the nucleoli the observation of the chromonemata is easy (Fig. 9). As the telophase advances, the nucleus increases in size, and the chromonema spirals become further expanded, ultimately to fill the entire nucleus



Figs. 6–14. From telophase to early mid-prophase. Explanation in the text.

in the form of expanded spirals or corrugated threads, and thus it becomes hardly possible to distinguish single chromosomes. In this stage no such case was observed as might confirm the finding of MARSHAK that the pair of chromatids (H_1) separate from one another except for the region of spindle fibre insertion. During the interval through telophase and interphase the chromonema

retains the spiral form, although the spirals become somewhat irregular. In rapidly dividing cells, the nuclei may pass on to the prophase without the intervention of a definite resting state.

Prophase. In the early stage of prophase, the chromonemata of every individual chromosome draw close together. Karyolymph separates the groups of chromonemata or the chromosomes from each another and thus individual chromosomes are rendered distinguishable from each another (Figs. 10, 11). Meanwhile the chromonemata are subjected to new spiralization and form regular spirals of the double-coiled type with major and minor coils (Figs. 12, 13). In the photomicrograph reproduced in Fig. 13, in which a part of the nucleus is shown photographed after it was broken by a strong pressure given on the cover glass, the new or small coils are clearly visible within the old or large coils (see also Fig. 3 in the critical review by KUWADA, 1939). As the prophase advances the minor coils grow in diameter, the major coils tending to be straightened out at the same time (Fig. 14), and the chromosomes are gradually shortened and thickened. During these stages twists are observed between the sister chromatids, which are usually eliminated before metaphase. As the stage advances, the longitudinal chromosome split becomes distinctly visible.

The straightening out of relic spirals, the shortening and thickening of the chromosomes, and the elimination of the twists between the sister chromatids have been explained by KUWADA & NAKAMURA (1933, 1934, 1935) as being connected with the new internal spiralization in the prophase and the subsequent increase in diameter of the new spirals, on the basis of BĚLAŘ's illustrations (1929) of the chromosome development in prophase; and a similar explanation is attempted by some other authors (SAX & SAX, 1935; SAX, 1935; NAITHANI, 1937; HUSTED, 1938; and others). The relic spirals may remain in the anaphase not being completely straightened out and form twists between the half-chromatids indicating the origin of the major spirals in meiosis (KUWADA, 1939; NEBEL, 1939).

In general, the discrepancy between fixation images is considerable when the fixing agents used are of different kinds[1]. Even in the case of the same treatment with the same agents, the chromosome structure may appear differently in different cells of the same tissue

1) In the preparation where the material is macerated with HCl after being fixed with the Bonn modification of FLEMMING's solution, the anaphase chromosomes present an alveolate structure or an apparently interlaced chromonema structure (Fig. 5). A similar figure has been obtained by MARSHAK (1936) as a result of a "slight modification of the fixation technique." He considers that if fixation breaks down the cylinders about which the smaller spirals are wound, only intertwined spirals would be evident.

(cf. Figs. 15a and b 16a and b). Accordingly, an absolute credence is not to be given without hesitation to certain fixation images, especially those obtained by such a complicated method of preparation as we used, they concern the fine structure of the chromosome. We may, however, from the results obtained in the present investigation, perhaps say that the following three points have been confirmed: 1) that anaphase chromosomes consist of two chromatids (half-chromosomes), each of which consists of a chromonema spiral or spirals, 2) that the new internal spiralization takes place in the prophase chromosome, and 3) that the statement of SAX & SAX (1935) that the old spirals are never straightened out before the new coiling takes place in the prophase, in other words, that the chromonema maintains a coiled aspect in all stages of its history, is reasonable.

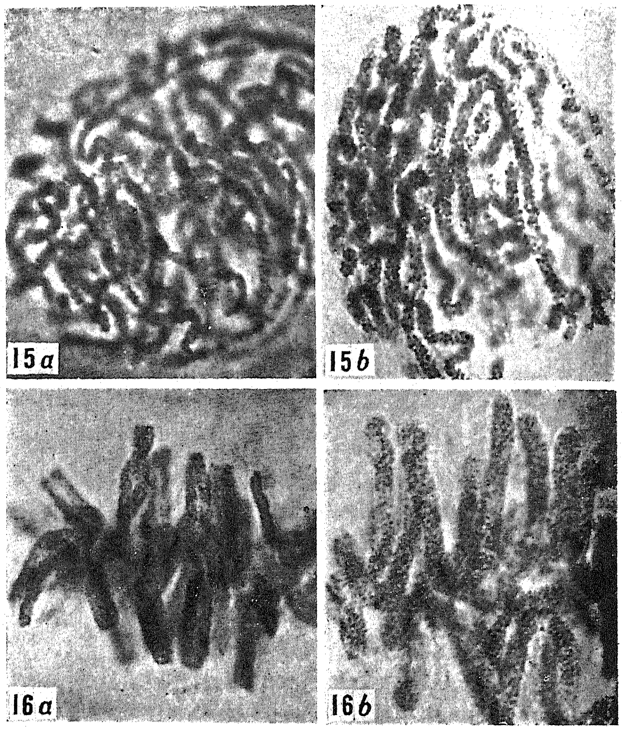


Fig. 15. Late mid-prophase. Fig. 16. Metaphase

Summary

Some fine structures of chromosomes which are not rendered visible by other methods, are shown to exist in the preparations made by the maceration method used in the present investigation.

Each anaphase chromosome is composed of two chromatids (half-chromosomes) each of which contains a chromonema spiral or spirals. The two chromatids may twist around each other to a greater or less degree. During telophase and interphase the chromonema remains in the coiled state, although the coiling is rendered somewhat irregular. At the commencement of prophase the chro-

monemata of each individual chromosome seem to draw close together. In the following stage the chromonema spirals are converted to regularly coiled major spirals in which the new or minor coiling is in progress, as the result of which the prophasic chromosome changes, such as the straightening out of the old spirals, and the thickening and shortening of the chromosome, take place. Gradually each chromosome develops a visible longitudinal split.

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Über den Golgiapparat und die Mitochondrien der Spermatogonien sowie Spermatozyten des Menschen, nebst Bemerkungen der Riesenspermatogonien

Von

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I. Einleitung

Der Formwechsel und das Schicksal des Golgiapparates und der Mitochondrien der sich entwickelnden Samenzellen sind von recht zahlreichen Autoren erforscht worden. Die Mehrzahl solcher Arbeiten befassen sich mit der Untersuchung der Spermatogenese an tierischem Material, so daß solche am Menschenhoden nahezu fehlt, bis neuerdings die Arbeit von GATENBY und BEAMS (1935) erschienen ist. Diese Autoren haben vorwiegend den Golgiapparat und die Mitochondrien der menschlichen Samenzellen in verschiedenen Entwicklungsstadien eingehend erforscht. AYKROYD (1938) hat in seiner zytologischen Untersuchung der menschlichen Oozyten auch den Golgiapparat der menschlichen Spermatogonien und Spermatozyten beiläufig kurz beschrieben. STIEVE (1930) hat die Veränderung der Mitochondrien bei Differenzierung der unentwickelten Samenzellen zu Spermatogonien und dann zu Spermatozyten bei Menschenmaterial eingehend verfolgt. Bei der Untersuchung der menschlichen Spermatogenese ist die genaue Unterscheidung der Spermatogonien und Spermatozyten, wie von ANDRES (1933), STIEVE (1930) u. a. angegeben, häufig schwer. Nach STIEVE sind die jüngsten Spermatozyten in ihrer Größe und in ihrem histologischen Bau nicht von den Spermatogonien verschieden. Deshalb

kann man mittels der gewöhnlichen, histologischen Beobachtung die Unterscheidung nicht mehr vornehmen.

Daß im Menschenhoden die Riesenzellen physiologisch vorkommen, ist seit langem bekannt. Neuerdings hat ANDRES (1933) die Riesenspermatogonien im Menschenhoden hauptsächlich von Standpunkt der Chromosomenforschung untersucht. Die Kenntnisse der Zytoplasmakomponente in den Riesenzellen sind heute noch mangelhaft.

In der vorliegenden zytologischen Untersuchung der menschlichen Spermatogenese[1] habe ich das Verhalten des Golgiapparates und der Mitochondrien in den Spermatogonien, Spermatozyten und Riesenzellen eingehend erforscht. Durch diese Untersuchung konnte ich den Übergang der Spermatogonien zu Spermatozyten zytologisch genau verfolgen.

II. Material und Methode

Das Untersuchungsmaterial besteht aus 5 lebendfrischen Hoden, welche bei unseren vorhergehenden zwei zytologischen Untersuchungen (ITO und OINUMA, 1939 und ITO und HIOKI, 1940) benutzt wurden. Die Untersuchungsmethoden sind auch ganz gleich; die sofort nach dem Tod herausgeschnittenen Gewebsstücke wurden für die Darstellung des Golgiapparates nach KOLATCHEV'scher Methode und für die der Mitochondrien mit LEVI'scher und CHAMPY'scher Flüssigkeit behandelt. Alle fixierte Gewebsstücke wurden in Paraffin eingebettet und in 4–5 μ dicke Serienschnitte zerlegt. Die KOLATCHEV-Präparate wurden ohne Färbung oder nach der KULL'scher Methode mit Fuchsin tingiert beobachtet, um Golgiapparat und Mitochondrien gleichzeitig darzustellen. LEVI- und CHAMPY-Präparate wurden mit HEIDENHAIN's Eisenhämatoxylin oder mit Anilinfuchsin nach KULL angefärbt.

Wir kommen hier der angenehmen Pflicht nach Herrn Dr. G. TAKAHASHI†, Vorstand des pathologischen Institutes des Mantetsu-Hospital zu Hsin King und Herrn Dr. K. KISO, Vorstand der Augenklinik desselben Hospital, für ihre freundliche Hilfe bei Ansammlung des Untersuchungsmaterials unseren herzlichsten Dank auszusprechen.

III. Eigene Befunde

1. Spermatogonien. Wie allgemein bekannt, sind die Spermatogonien in der Regel auf der Innenfläche der Membrana propria

1) Diese Abhandlung ist die dritte zytologische Untersuchung des Menschenhodens; in der ersten haben wir die Zwischenzellen (ITO u. OINUMA [1939]) und in der zweiten die Sertolizellen (ITO u. HIOKI [1940]) beschrieben.

des Samenkanälchens nahezu in einer Reihe angeordnet. Ihre der Membrana propria anliegende Fläche ist haüfig mehr oder weniger eben und breit, indem ihr Zelleib häufig entlang der Eigenhaut etwas gestreckt sind. Ihre Gestalt ist somit meistens nicht kreisrund (Fig. 1–6). Dagegen ist ihr Kern immer rundlich gestaltet; bei LEVI-, CHAMPY- und KOLATCHEV-Präparaten ist er homogen gebaut und sieht intensiv gefärbt mehr dunkler aus. Die Kernkörperchen sind in der Regel rundlich, kommen meistens mehrfach vor. Sie liegen häufig auf der Kernmembran angeschmiegt. Das Zytoplasma der Spermatogonien ist im allgemeinen hell, aber der perinukleäre Teil ist immer schwach granuliert und sieht dunkel aus; dieses granulierte Zytoplasma schickt die Fortsätze nach der Peripherie, so daß das Zytoplasma der Spermatogonien oftmals grob wabig erscheint.

Die Größe des Kerns der gewöhnlichen Spermatogonien variiert zwischen 6 μ und 8.5 μ. Die Kerngröße von etwa 6.7 μ ist am häufigsten.

a. *Golgiapparat der Spermatogonien.* Der Golgiapparat der Spermatogonien des Menschen ist bis jetzt nur von GATENBY und BEAMS (1935) beschrieben worden. Nach meiner Beobachtung ist der Golgiapparat der menschlichen Spermatogonien anders gebaut als der der gewöhnlichen somatischen Zellen der Wirbeltiere. Er stellt nicht das Netz („komplexe Form" nach HIRSCHLER) dar und besteht aus den zahlreichen diskreten kleinen Elementen, wie es bei den Zellen der wirbellosen Tiere der Fall ist. GATENBY und BEAMS (1935) haben ebenfalls bei den menschlichen Spermatogonien aufmerksam gemacht, daß der Golgiapparat dieser Zellen aus zahlreichen osmiophilen Blöckchen besteht. Gleiches Verhältnis ist bei den Spermatogonien der niederen Tiere zu finden. Nach der neueren Arbeit von SOKOLOW (1926) zeigt der Golgiapparat der Spermatogonien der Pseudoskorpione „diffusen Charakter"; er setzt sich nämlich aus einer wechselnden Anzahl von einzelnen Elementen zusammen, welche gewöhnlich im Zytoplasma zerstreut liegen.

Also gehört der Golgiapparat der menschlichen Spermatogonien zu der „diffusen Form" nach HIRSCHLER. Die einzelnen Apparatelemente stellen kleine rundliche, halbmondförmige, stäbchenförmige u.s.w. geformte Körperchen dar, welche durch Osmierung schwarz imprägniert werden (Fig. 1, 2). Bei näherer Beobachtung mit stärkerer Vergrößerung setzen sich die einzelnen Elemente wieder aus dem intensiv schwarz imprägnierten Externum und dem schwach imprägnierten Internum zusammen. Also stellen sie „Golgi-System" nach HIRSCH (1939) dar. Aber unter den System bildenden Apparatelementen gibt es kleine Körnchen, welche im ganzen stark

osmiert worden sind; diese stellen nach der Meinung von HIRSCH die „Präsubstanz“ dar. Wir müssen aber bei der Unterscheidung der Präsubstanz von den Golgi-Systemen sehr vorsichtig sein, weil die letzteren oft optisch gleichmäßig tiefschwarz imprägnierte Körperchen täuschen können.

Die Tatsache, daß der Golgiapparat der menschlichen Spermatogonien die „diffuse Form“ darlegt, ist interessant. Nach den zytologisch-entwicklungsgeschichtlichen Untersuchungen der Autoren zeigt der Golgiapparat der Zellen der Wirbeltiere in der embryonalen Zeit eine „diffuse Form“ (FANANAS (1922), KWAN (1936, '37) u.a.) Also entsteht meines Erachtens die Annahme, daß die Spermatogonien nach dem Charakter ihres Golgiapparates als die in einem nicht genug differenzierten, im embryonalen Zustand stehenden Zellen zu betrachten sein dürfen.

Die Apparatelemente verteilen sich den Kern umgebend in der unmittelbaren Nähe der Kernmembran. Diese perinukleäre

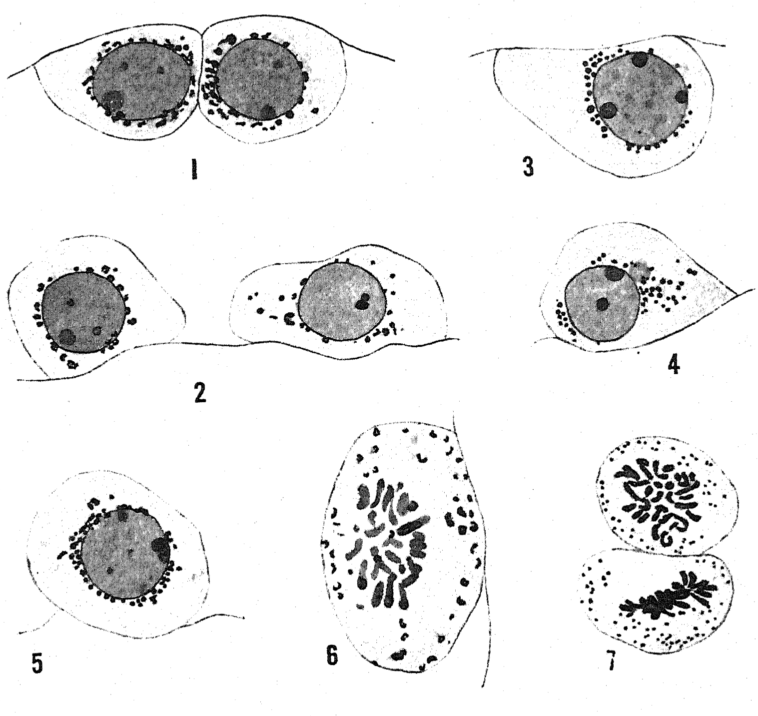

Fig. 1–7. Spermatogonien. 1–2 Golgiapparat der Spermatogonien. Imprägnation nach KOLATCHEV. 3–4. Mitochondrien der Spermatogonien. CHAMPY-KULL. 5. Gleichzeitige Darstellung des Golgiapparates und der Mitochondrien im Spermatogonium. KOLATCHEV-KULL. 6. Golgiapparat bei der Spermatogonienteilung. Imprägnation nach KOLATCHEV. 7. Mitochondrien bei der Spermatogonienteilung. CHAMPY-KULL. Vergr. etwa 1650 fach.

Verteilung ist aber nicht gleichmäßig; der größere Teil der Elemente kann sich mitunter auf einem Pol des Kerns ansammeln. Bei den entlang der Membrana propria gestreckten Zellen breiten die Apparatelemente auch in der gleichen Richtung nach der Peripherie der Zelle aus (Fig. 2).

b. *Mitochondrien der Spermatogonien*: Nach STIEVE (1930) sind die Mitochondrien der menschlichen Spermatogonien grobkörnig, während GATENBY und BEAMS (1935) sie als klein körnchenartig bezeichnet haben. Nach der Beschreibung von KATES (1938) sind die Mitochondrien der Spermatogonien des Meerschweinchens granulär oder kurz stäbchenförmig. Nach meiner eigenen Beobachtung sind die Mitochondrien der menschlichen Spermatogonien fast immer grobkörnig, wie von STIEVE (1930) angegeben. Doch mischen sie in wechselnder Zahl kleinere Granula. Die Anordnung ist ganz gleich wie die des Golgiapparates; sie sind auf der Kernmembran verteilt den ganzen Umfang des Kerns umgebend (Fig. 3). Sie bilden aber mitunter auf einem Pol des Kerns mehr oder weniger deutliche Anhäufung, welche sich oft nach der Peripherie mehr oder weniger weit ausdehnt (Fig. 4).

Zwecks der gleichzeitigen Darstellung des Golgiapparates und der Mitochondrien habe ich die nach KOLATCHEV behandelten Schnitte mit Anilinfuchsin-Aurantia-Färbung nach KULL tingiert. Bei solchen Präparaten war es klar dargelegt, daß die einzelnen schwarz imprägnierten Apparatelemente und die mit Fuchsin rot gefärbten Mitochondrien sich miteinander beimengt perinukleär befinden, wie Fig. 5 gezeigt.

c. *Golgiapparat und Mitochondrien bei der Spermatogonienteilung*. Das Verhalten des Golgiapparates und Chondrioms bei der mitotischen Zellteilung ist bei verschiedenen Zellarten von verschiedenen Autoren auseinandergesetzt worden (PERRONCITO 1911, DEINEKA, 1912 u.a.). Dabei zersetzt sich der Golgiapparat in zahlreiche Körperchen, Diktyosomen nach PERRONCITO, welche wahrscheinlich den Apparatelementen entsprechen, und in nahezu gleicher Zahl in die Tochterzellen übergehen, um dort wieder den Golgiapparat zu bilden. Die Mitochondrien werden ebenfalls zur Hälfte in Tochterzellen verteilt.

Weil bei den menschlichen Spermatogonien der Golgiapparat eine diffuse Form ist, so stellen die einzelnen Apparatelemente bei der mitotischen Teilung ohne weiteres die Diktyosomen dar. In der Metaphase ordnen sich die Apparatelemente zum größten Teil in der Peripherie des Zellkörpers nahezu gleichmäßig an (Fig. 6). Wir können an den einzelnen Apparatelementen die osmiophile und osmiophobe Substanz, nämlich Externum und Internum nach HIRSCH

gut unterscheiden, somit trifft man hier „Golgi-Systeme" nach Hirsch, aber nicht „Präsubstanzen" nach demselben an. Bei der Anaphase werden die Apparatelemente nahezu in gleicher Zahl in die Tochterzellen verteilt.

Gleiche Verhältnisse bemerkt man auch an den Mitochondrien der sich teilenden Spermatogonien; bei der Metaphase finden sie sich nahezu ausschließlich in der Peripherie des Zytoplasma. Dabei soll man nicht außer Acht lassen, daß die einzelnen körnchenförmigen Mitochondrien viel kleiner als die der Spermatogonien im Ruhestadium sind. Es ist also wahrscheinlich, daß die einzelnen grobkörnigen Mitochondrien der Spermatogonien sich bei der Mitose in zwei kleineren Körnchen teilen.

2. **Spermatozyten.** Die Spermatozyten in verschiedenen Entwicklungsstadien finden ihren Platz in dem Samenepithel in den Spermatogonien nach innen anschliessenden Reihen, so daß sie in der Regel von der Membrana propria mehr oder weniger entfernt liegen. Die Gestalt der Spermatozyten ist rundlich oder elliptisch, der Kern ist in der Regel rundlich geformt. Die Größe der Spermatozyten schwankt in gewissem Maße, weil diese Zellen in dem Wachstumsstadium der Samenzellen sich befinden. Die ausgewachsenen Spermatozyten besitzen einen Kern von 8.5–9.5 μ Größe. Die Innenstruktur des Kerns ist nach dem Stadium der Meiosis verschieden, wie in den Figuren der Spermatozyten leicht ersehen. Im allgemeinen sieht der Kern etwas heller als der der Spermatogonien aus; wir finden meistens darin ein gut ausgeprägtes großes rundliches Kernkörperchen, welches sich von den einigen Nebennukleolen durch die ausgezeichnete Größe leicht unterscheiden läßt. Das Zytoplasma der Spermatozyten sieht homogen und viel dunkler als das der Spermatogonien aus, weil es viel dichter gebaut ist.

a. *Golgiapparat der Spermatozyten.* Der Golgiapparat der Spermatozyten des Menschen ist bis heute nur von Gatenby und Beams (1935) beobachtet worden. Nach ihren Beschreibungen stellt er ein einfacher rundlicher Körper auf der Kernmembran dar, welcher infolge der Sammlung der einzelnen osmiophilen Elementen in einer Area gebildet worden ist. Diese Area stimmt nach ihrer Angabe mit der Sphäre (Idiozom) mit Zentriol überein. Sie haben dabei als eine Eigentümlichkeit der menschlichen Spermatozyten aufmerksam gemacht, daß diese mitunter außer dem eben genannten Hauptgolgiapparat noch einen accessorischen, kleineren Golgiapparat führen können. Meine Ergebnisse bezüglich des Golgiapparates der menschlichen Spermatozyten stimmen im großen ganzen mit denjenigen von Gatenby und Beams überein. Ich konnte in diesen Zellen nicht mehr den Golgiapparat von einer „diffusen Form" wie

in den Spermatogonien wahrnehmen. Bei den ausgewachsenen Spermatozyten habe ich immer, wie in der Fig. 8 ersichtlich, den knäuelartig gebauten Golgiapparat gefunden, an welchem man nicht mehr einzelne Apparatelemente unterscheiden kann. Also handelt es sich hier um den Golgiapparat von einer „komplexen Form“ oder um „Polysystem“ nach HIRSCH. Der Golgiapparat der ausgewachsenen menschlichen Spermatozyten stellt einen, im ganzen leicht schwarz osmierten, rundlichen oder kappenförmigen Körper dar, welcher auf einem Pol des Kerns sitzt. Wie von GATENBY und BEAMS berichtet, habe ich auch in den menschlichen Spermatozyten oft außer dem Hauptgolgiapparat noch einen oder zwei kleinere accessorische Apparate gefunden (Fig. 9), welche gleich gebaut sind wie der erstere. Der im ganzen schwach schwarz osmierte Golgiapparat weist innen eine Knäuelstruktur auf, welche durch die stark schwarz osmierten, gebogenen Fäden oder Stücke gebildet ist. Durch Zusammenfließen des osmiophilen Externum und osmiophoben Internum der einzelnen Apparatelemente ist diese Struktur wahrscheinlich zustandegekommen. Hier ist auch der Golgiapparat durch die osmiophile Substanz umsäumt.

b. *Mitochondrien der Spermatozyten.* Nach STIEVE (1930) liegt die Mehrzahl der Mitochondrien der Spermatozyten des Menschen in der unmittelbaren Nähe des Kerns, einzelne finden sich aber immer in der ganzen Zelle verstreut. GATENBY und BEAMS (1935) kamen auch bei den Spermatozyten des Menschen nahezu den gleichen Ergebnissen. Nach meiner eigenen Beobachtungen sind die Mitochondrien der menschlichen Spermatozyten sämtlich körnchenförmig; sie sind aber viel kleiner als die der Spermatogonien (Fig. 10). Sie sind im ganzen Zytoplasma, das Ektoplasma ausgenommen, verteilt. Ihre Zahl ist beträchtlich. In den KOLATCHEV-Präparaten, welche nach KULL'scher Methode gefärbt sind, kann man Mitochondrien gleichzeitig mit dem Golgiapparat beobachten. Zwischen beiden Gebilden entsteht keine besondere Beziehung (Fig. 11). Bei den nach CHAMPY oder LEVI fixierten und mit KULL'scher Methode oder mit Eisenhämatoxylin nach HEIDENHAIN gefärbten Präparaten konnte ich in den Spermatozyten an Stelle des Golgiapparates eine rundliche, etwas dunkel gefärbte Area auf einem Pol des Kerns vorfinden (Fig. 10), welche, wie von GATENBY und BEAMS angegeben, Sphäre oder Idiozom ist. Darin habe ich aber oftmals die Zentralkörperchen vermisst. Nach diesen Autoren enthält das Idiozom kleine Vakuolen mit einem sehr kleinen Körnchen, „Pro-acrosomic-granules“. Nach meiner Beobachtung sieht das Idiozom ganz homogen aus, enthält keine solche Gebilde. Zwischen dem Idiozom und den Mitochondrien besteht auch keine

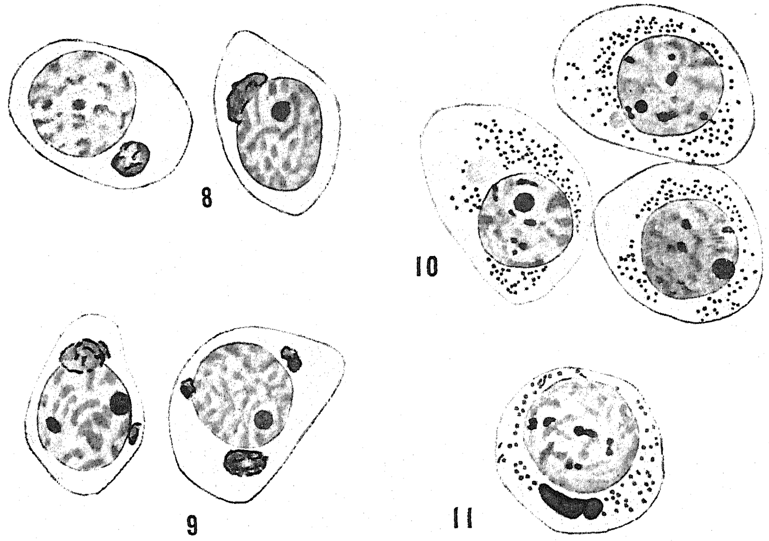

Fig. 8–11. Ausgewachsene Spermatozyten. 8–9. Golgiapparat der Spermatozyten. Imprägnation nach KOLATCHEV. 10. Mitochondrien und Idiozom der Spermatozyten. CHAMPY-KULL. 11. Gleichzeitige Darstellung des Golgiapparates und der Mitochondrien im Spermatozyt. KOLATCHEV-KULL. Vergr. etwa 1650 fach.

besondere Beziehung. Das Wesen des Idiozoms ist vielfach erörtert. Nach meiner Ansicht sind das Idiozom und die osmiophobe Substanz des Golgiapparates nicht identische Bildung. Das erstere mit dem Zentralkörperchen ist, wie mir scheint, von dem Golgiapparat umhüllt. Diese Vermutung wird von der folgenden Tatsache unterstützt, daß das Idiozom immer kleiner als der Golgiapparat ist. Wie in der Fig. 4. ersichtlich, habe ich selten in den Spermatogonien auch rundlich geformte Sphäre oder Idiozom wahrgenommen, in welchen, wie oben gesagt, kein komplexer Golgiapparat sich befindet. Somit seien die in den Spermatozyten bemerkten Verhältnisse des Golgiapparates und Idiozomes höchstwahrscheinlich folgendermaßen erfolgt, daß die Apparatelemente des diffusen Golgiapparates der Spermatogonien beim Übergang der letzteren zu Spermatozyten auf dem Idiozom konzentrisch angeordnet werden, um dort einen komplexen Golgiapparat zu bilden. Auf diese Weise entsteht in den Spermatozyten sogenannter Golgi-Idiozom-Komplex. Also stimmen Golgiapparat und Zentralkörperchen hier topographisch überein.

3. **Jüngere Spermatozyten (Übergang der Spermatogonien zu Spermatozyten).** Aus obigen Ergebnissen kann man gewöhnlich die Spermatogonien von den Spermatozyten leicht unterscheiden. In der vorliegenden Untersuchung habe ich häufig solche Zellen begegnet, bei welchen diese Unterscheidung äußerst schwer ist (Fig. 12, 13,

14, 15). Solche Zellen sind im großen ganzen den kleinen Spermatogonien ähnlich, doch sind sie in verschiedenen Punkten von den letzteren verschieden. Sie befinden sich gewöhnlich von der Membrana propria der Samenkanälchen etwas nach innen entfernt, aber es ist nicht selten, daß sie auf der letzteren zwischen den Spermatogonien liegen. Diese Zellen sind klein; die Größe des Kerns ist nahezu gleich wie bei den kleinen Spermatogonien, indem sie sich auf etwa 6–6.5 μ beläuft. Der Kern sieht dunkel und homogen aus, wie bei den Spermatogonien; das Kernkörperchen ist rundlich und groß, liegt in der Nähe der Kernmitte, in dieser Hinsicht ähneln sie den Spermatozyten. Das Zytoplasma ist, wie bei den letzteren, dunkel und homogen. Aus obigen Befunden kann man nicht mit Sicherheit entscheiden, ob diese Zellen den Spermatogonien oder den Spermatozyten gehören sollen.

Die Struktur des Golgiapparates dieser Zellen ist sehr interessant. Er ist von dem diffusen Golgiapparat der Spermatogonien in gewissem Masse verschieden. Wir finden in diesen Zellen die auf dem ganzen Umfang des Kerns verteilten Apparatelemente nicht mehr; diese sammeln sich, wie in der Fig. 12 gesehen, auf einem Pol des Kerns kappenartig. Dort fließen sie allmählich miteinander zusammen und bieten die in der Fig. 13 gezeigte Struktur dar; schließlich bilden sie auf einem Pol des Kerns einen rundlichen Golgiapparat aus, welcher zweifelsohne eine komplexe Form darstellt und ganz gleich wie der Golgiapparat der Spermatozyten gebaut ist (Fig. 14). In diesen Zellen kann man somit den Übergang des Golgiapparates von diffuser Form zu komplexer Form gut verfolgen. Aus den Befunden des Golgiapparates kann man mit Sicherheit feststellen, daß diese kleinen Zellen die jüngsten Spermatozyten sind, welche soeben durch die letzte Spermatogonienteilung gebildet worden sind. Nach der letzten Spermatogonienteilung werden die Tochterzellen als Spermatozyten bezeichnet, darin sammeln sich die von den Spermatogonien unmittelbar eingeführten Apparatelemente auf einem Pol des Kerns um das Idiozom, um dort einen komplexen Golgiapparat zu bilden. In den jüngsten Spermatozyten kann man also die Entstehungsweise des komplexen Golgiapparates aus den einzelnen Apparatelementen gut verstehen. Der Golgiapparat von komplexem Typus, worin man die einzelnen Apparatelementen in der Regel nicht mehr klar unterscheiden kann, ist wesentlich aus den letzteren zusammengesetzt. Also kann man sagen, daß die Apparatelemente, welche aus dem osmiophilen Externum und osmiophoben Internum zusammengesetzt worden sind, nichts anders als die Bausteine nämlich die Struktureinheiten des Golgiapparates sein sollen. Aus der obigen Schluß-

folgerung läßt sich die Beziehung zwischen dem komplexen und diffusen Typus des Golgiapparates gut verstehen.

Die Mitochondrien dieser jüngsten Spermatozyten sind ganz gleich beschaffen wie die ausgewachsenen Spermatozyten, obwohl ihre Zahl viel weniger als bei den letzteren. In den jüngsten Spermatozyten sind die Mitochondrien klein und granulär, verstreuen sich im ganzen Zytoplasma (Fig. 15). In den mit KULL'scher Methode gefärbten KOLATCHEV-Präparaten kann man die Lageverhältnisse der Mitochondrien und des Golgiapparates beobachten (Fig. 16).

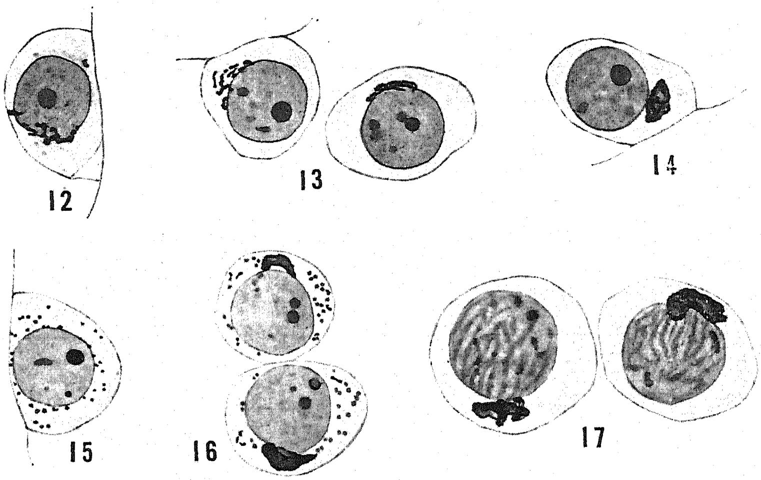

Fig. 12–17. Jüngere Spermatozyten. 12–14. Golgiapparat der jüngsten Spermatozyten. Imprägnation nach KOLATCHEV. 15. Mitochondrien des jüngsten Spermatozytes. CHAMPY-KULL. 16. Gleichzeitige Darstellung des Golgiapparates und der Mitochondrien in den jüngsten Spermatozyten. KOLATCHEV-KULL. 17. Golgiapparat der Spermatozyten im Synapsis-Stadium. Imprägnation nach KOLATCHEV. Vergr. etwa 1650 fach.

Durch diese zytologische Untersuchungsmethode ist die Unterscheidung der Spermatozyten und Spermatogonien jedes Entwicklungsstadium hindurch nicht schwer und kann man den Übergang der letzteren zu den ersteren eingehend verfolgen.

Mit dem Beginn des Wachstums der jüngsten Spermatozyten vergrößert sich auch der Golgiapparat. In dem Kern gehen nun die Erscheinungen der Reduktionsteilung vor sich. In der Fig. 17 findet man zwei Spermatozyten im Stadium von Chromosomensynapsis; der Golgiapparat, welcher das Idiozom mit Zentralkörperchen im Innern beherbergt, lagert sich in dem Pol, wohin die Schenkel der Schleifen (Chromosomen) sich sämtlich konvergieren.

4. **Riesenspermatogonien.** Daß in den Samenkanälchen der Hoden die Riesenzellen physiologisch vorkommen, ist durch die

Untersuchungen der verschiedenen Autoren nachgewiesen worden. Nach ANDRES (1933) wurden Riesenzellen in Menschenhoden zuerst von WIDERSPERG (1885) beschrieben. Die Riesenspermatogonien wurden von DUESBERG (1906), WINIWARTER (1912), PAINTER (1923), TAKAGI (1925), SHIINA (1925), WINIWARTER und OGUMA (1926), EVANS und SWEZY (1929) u.a. beobachtet. Neuerdings wurden sie von ANDRES (1933) sehr eingehend und vorwiegend chromosomologisch untersucht. Er hat die Riesenspermatogonien in zwei Arten eingeteilt, in einkernigen und mehrkernigen, und das weitere Schicksal dieser Zellen verfolgt. STIEVE (1930) hat auch in seinem Handbuchbeitrag die gleiche Einteilung vorgenommen. Er bezeichnet dabei die einkernigen Riesenspermatogonien als „männliche Eier" bezeichnet. Nach meiner Meinung fehlen aber heute noch morphologische Befunde, welche mit aller Sicherheit nachweisen, daß die von Autoren als Riesenspermatogonien genannten Zellen in der Wirklichkeit zu den Spermatogonien gehören sollen. Nach den obigen zytologischen Beobachtungen sind die Spermatogonien und Spermatozyten in der Beschaffenheit des Golgiapparates und der Mitochondrien gänzlich verschieden. Also ist es zweckmäßig, daß man für die Entscheidung des Wesens dieser sogenannten Riesenspermatogonien die Zytoplasmakomponente genau untersucht.

In der vorliegenden Untersuchung habe ich in den Samenkanälchen der gesamten untersuchten Hoden immer nicht selten die Riesenzellen bemerkt, welche immer auf der Innenfläche der Membrana propria zwischen den gewöhnlichen Spermatogonien sich finden. Der Kern dieser großen Zellen ist im allgemeinen dunkel und fast homogen gebaut, der Kernkörperchen kommt mehrfach vor und liegt häufig auf der Kernmembran. Das Zytoplasma ist im allgemeinen hell und locker gebaut, sieht mitunter grobwabig aus. Die perinukleäre Zone ist aber dicht gebaut und erscheint dunkel. Die Lage und die Beschaffenheiten des Kerns sowie Zytoplasma dieser Zellen sprechen also dafür, daß diese Zellen höchstwahrscheinlich zu den Spermatogonien gehören. Wie von STIEVE (1930), ANDRES (1933) u. a. angegeben, konnte ich auch einkernige und mehrkernige Riesenspermatogonien bemerken, welche beide nahezu in gleicher Häufigkeit vorkommen.

Bei den einkernigen Riesenspermatogonien (Fig. 18, 19) schwankt die Kerngröße beträchtlich. Der größte Kern beträgt etwa 16 μ. Die Unterscheidung der kleineren Riesenzellen von den gewöhnlichen Spermatogonien ist häufig schwer. Ich habe solche Zellen, deren Kern größer als 9.5 μ ist, in die Kategorie der Riesenzellen gereiht, weil solche Zellen sich schon beim Anblick mit der

schwächeren Vergrößerung durch ihre Größe kennzeichnen (Fig. 18). Der Golgiapparat und die Mitochondrien der einkernigen Riesenspermatogonien sind ganz gleich beschaffen wie die gewöhnlichen

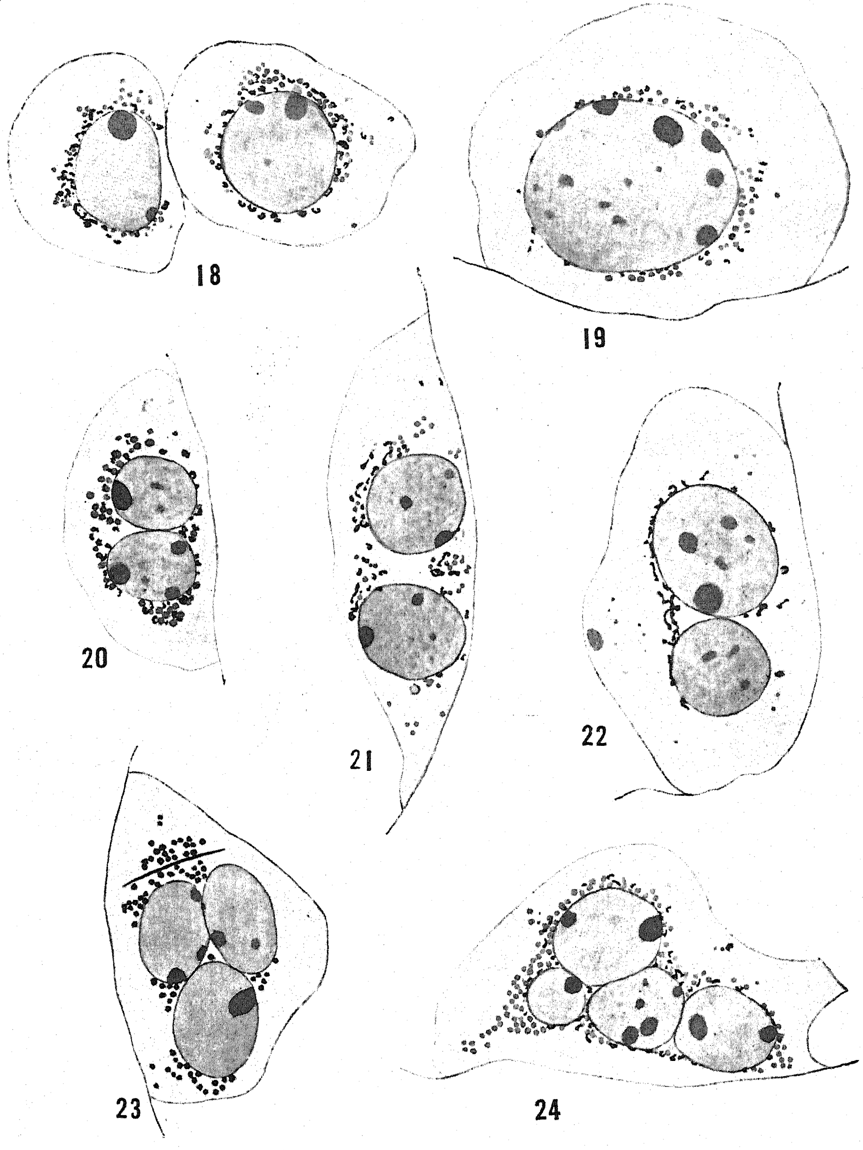

Fig. 18–24. Riesenspermatogonien. 18–19. Golgiapparat und Mitochondrien der einkernigen Riesenspermatogonien. KOLATCHEV-KULL. 20–21. Golgiapparat und Mitochondrien der zweikernigen Riesenspermatogonien. KOLATCHEV-KULL. 22. Golgiapparat des zweikernigen Riesenspermatogonium. Imprägnation nach KOLATCHEV. 23. Mitochondrien und Kristalloid des dreikernigen Riesenspermatogonium. CHAMPY-KULL. 24. Golgiapparat und Mitochondrien des vierkernigen Riesenspermatogonium. KOLATCHEV-KULL. Vergr. etwa 1650 fach.

Spermatogonien. Die in der Fig. 18 und 19 abgebildeten Zellen stammen aus den KOLATCHEV-Präparaten, welche mit KULL'scher Methode gefärbt sind. In diesen einkernigen Riesenspermatogonien sind somit Golgiapparat und Mitochondrien gleichzeitig dargestellt. Der Golgiapparat besteht aus den einzelnen Apparatelementen, welche perinukleär diffus verteilt sind (diffuse Form). An den Apparatelementen kann man osmiophiles Externum und osmiphobes Internum unterscheiden. Die Mitochondrien sind grobgranulär und verteilen sich, mit den Apparatelementen beimengt, auf der Kernmembran. Aus den obigen zytologischen Befunden kann man mit aller Sicherheit sagen, daß die erwähnten Riesenzellen zu den Spermatogonien gehören.

Die mehrkernigen Riesenspermatogonien sind häufig unregelmäßig geformt, strecken sich meistens entlang der Membrana propria, so daß sie mit ihrer breiten Fläche der letzteren sich anlegen. Die mehrkernigen Riesenzellen werden wieder in zweikernige und mehr als zweikernige unterschieden. Die Kerngröße der zweikernigen Riesenzellen ist bald von gewöhnlichen Größe (Fig. 20, 21), bald mehr oder weniger größer als 9.5 μ (Fig. 22), wie bei den einkernigen Riesenspermatogonien. Die zwei Kerne der zweikernigen Riesenzellen sind bald gleichgroß (Fig. 20, 21), bald aber ungleichgroß (Fig. 22). In der Mehrzahl der Fälle finden sich die beiden Kerne in der Zellmitte nebeneinander dicht zusammengedrängt; zuweilen liegen sie mehr oder weniger voneinander entfernt (Fig. 21).

Die drei bis mehrkernigen Riesenzellen sind viel seltener als die zweikernigen getroffen. Sie sind immer beträchtlich groß; die Kerne liegen immer in der Zellmitte miteinander dicht zusammengedrängt (Fig. 23, 24). Die Kernzahl beträgt nach meiner Beobachtung höchstens 6. Die Größe der einzelnen Kerne ist gewöhnlich und nahezu gleichgroß, oft kleineren Kern beimengt (Fig. 24).

Die Beschaffenheiten des Golgiapparates und der Mitochondrien der zwei und mehrkernigen Riesenzellen sind ganz gleich wie die gewöhnlichen Spermatogonien. Der Golgiapparat gehört zu der diffusen Form; die Mitochondrien sind grobgranulär. Die Apparatelemente und Mitochondrien sind miteinander beimengt perinukleär angeordnet. Aus den Befunden dieser Zytoplasmakomponente gehören die mehrkernigen Riesenzellen zweifelsohne zu den Spermatogonien.

Die einkernigen und mehrkernigen Riesenzellen enthalten oft das lange, nadelförmige, an beiden Enden zugespitzte Kristalloid, welches den Spermatogonien eigentümlich ist (Fig. 23). Es ist

bemerkenswert, daß dieses Spermatogonienkristalloid bei den mehrkernigen Riesenspermatogonien immer einfach vorkommt, wie bei den gewöhnlichen Spermatogonien.

Die Genese der Riesenspermatogonien ist mir unklar; es scheint mir aber möglich, daß die zwei und mehrkernigen Riesenspermatogonien infolge Zusammenfließen der zwei oder mehreren Spermatogonien zustandekommen.

Das Schicksal der einkernigen und mehrkernigen Spermatogonien hat ANDRES (1933) eingehend auseinandergesetzt; nach seiner Angabe werden die einkernigen Riesenspermatogonien zu den Riesenspermien mit Diploidchromosomenzahl, und von den mehrkernigen Riesenspermatogonien entstehen wahrscheinlich mehrköpfige Spermien. Ich kann hier darüber nichts Sicheres äußeren.

Ich habe in der vorliegenden Untersuchung die einkernigen und zweikernigen Riesenspermatozyten wahrgenommen, welche den Golgiapparat von komplexem Typus enthalten. An den zweikernigen Riesenspermatozyten sind zwei Arten unterschieden, bei der einen haben zwei Kerne einen Golgiapparat gemein, bei der anderen haben sie je einen besonderen Golgiapparat.

IV. Zusammenfassung

In der vorliegenden Untersuchung habe ich bei den fünf gesunden Hoden, welche aus fünf Hingerichteten im lebendfrischen Zustand entnommen wurden, die Zytoplasmakomponente der Spermatogonien, Spermatozyten und Riesenzellen eingehend erforscht. Die wichtigen Ergebnisse sind im folgenden angegeben.

1) Die Spermatogonien liegen auf der Membrana propria der Samenkanälchen. Das Zytoplasma ist hell und grob wabig; der rundliche Kern von etwa 6–8.5 μ Größe sieht dunkel und homogen aus. Das Kernkörperchen kommt in der Regel mehrfach und häufig auf der Kernmembran vor. Der Golgiapparat ist von „diffuser Form"; die einzelnen Apparatelemente, welche aus dem osmiophilen Externum und osmiophoben Internum zusammengesetzt sind, sind perinukleär angeordnet. Die Mitochondrien sind grob granulär, finden sich mit den Apparatelementen beimengt in der perinukleären schmalen Zytoplasmazone.

2) Die ausgewachsenen Spermatozyten befinden sich in der Regel von der Membrana propria nach innen entfernt. Das Zytoplasma ist dicht und homogen gebaut. Der Kern von etwa 8.5–9.5 μ Größe enthält in der Nähe der Mitte einen großen Nukleolus. Der Golgiapparat ist von „komplexer Form," stellt auf einem Pol des Kerns einen rundlichen oder kappenförmigen Körper dar, welcher

im Innern ein rundliches Idiozom mit Zentralkörperchen beherbergt (Golgi-Idiozom-Komplex). Außer diesem Hauptgolgiapparat treten oft in menschlichen Spermatozyten 1–2 akzessorische Golgiapparate auf. Die Mitochondrien sind fein granulär und im großen ganzen diffus im Zytoplasma verteilt.

3) Auf der Membrana propria oder davon nach innen etwas entfernt kommen kleine Zellen mit einem rundlichen Kern von etwa 6–6.5 μ Größe vor, welche den kleinen Spermatogonien ähnlich aussehen. Durch die gewöhnlichen histologischen Beobachtungen kann man kaum entscheiden, ob diese Zellen zu den Spermatogonien oder Spermatozyten zuzurechnen seien. In diesen Zellen ist der Golgiapparat nicht mehr der diffusen Form; die Apparatelemente sammeln sich auf einem Pol des Kerns in eine kappenartige Figur an und beginnen miteinander zusammenzufließen, um einen komplexen Körper wie der Golgiapparat der ausgewachsenen Spermatozyten zu bilden. Die Mitochondrien sind fein granulär und im ganzen Zytoplasma zerstreut verteilt. Aus obigen zytologischen Befunden kann man mit Sicherheit sagen, daß diese Zellen die jüngsten Spermatozyten vertreten, welche durch die letzte Spermatogonienteilung gebildet worden sind. Man kann bei diesen Zellen den Übergang der Spermatogonien zu Spermatozyten genau verfolgen. Der Golgiapparat von komplexer Form ist also aus den Apparatelementen der diffusen Form zusammengesetzt, obwohl man darin kaum mehr die einzelnen Apparatelemente unterscheiden kann. Aus dieser Schlußfolgerung kann man umgekehrt sagen, daß die Apparatelemente die Bausteine oder Struktureinheiten des Golgiapparates seien.

4) In den Samenkanälchen des menschlichen Hodens kommen physiologisch nicht selten die Riesenzellen vor. Riesenzellen, welche nach den Beschaffenheiten des Kerns und Zytoplasma wahrscheinlich zu den Spermatogonien zuzurechnen sein dürfen, liegen immer auf der Membrana propria. Der Golgiapparat und die Mitochondrien solcher Zellen sind ganz gleich beschaffen wie bei den gewöhnlichen Spermatogonien. Daraus konnte ich feststellen, daß diese Riesenzellen nichts anders als die Riesenspermatogonien sind. Sie werden wieder in einkernige und mehrkernige Riesenzellen eingeteilt. Die Größe des Kerns der einkernigen Riesenzellen beträgt 9.5–16 μ. Die Kernzahl der mehrkernigen Riesenzellen ist 2–6. Außer den Riesenspermatogonien kommen im Samenepithel Riesenspermatozyten vor, welche einkernig oder zweikernig sind. Der Golgiapparat ist der komplexen Form wie bei den gewöhnlichen Spermatozyten. Bei zweikernigen Riesenspermatozyten besitzen die zwei Kerne bald einen Golgiapparat gemein, bald je einen besonderen Golgiapparat.

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Cytogenetic Studies in Artificially Raised Interspecific Hybrids of *Papaver*. VIII. F_1 plants of *P. bracteata* × *P. lateritium*.[1]

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(With 22 Text Figures)

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Introduction

In previous investigations, I have shown that neither of the plants *P. bracteata* and *P. lateritium* have any chromosome capable of association with those of *P. somniferum* in the PMCs of either F_1 plant *somniferum* × *bracteata* or *somniferum* × *lateritium* (YASUI 1927, 1937).

Between *P. bracteata* and *P. lateritium* I have succeeded in making a cross when *bracteata* was used as the female plant (Fig. 1), while the reciprocal crossing, *lateritium* × *bracteata*, has not yet been successful.

In the present inquiry I have endevoured to find on one hand the genetic relationship between the parent plants themselves and on the other to determine whether these parents have such chromosomes as are capable of association between themselves in the meiosis of the PMCs of the F_1 plants, and also to study the behaviour of such chromosomes in their meiosis, especially of univalents if present.

Material and Method

The behaviour of the chromosome in the PMCs was studied with either aceto-carmine smear material or that fixed with NAVASHIN's fluid. Paraffin sections were stained after NEWTON's gentian violet method and also with HEIDENHAIN's iron-alum heamatoxylin. Photomicrographs were taken with Zeiss 1/12 H.I.×100 and Leitz peripl. oc.×10. All drawings were made by the use of a camera lucida with Zeiss 1/12 H.I.×100 obj. and Komp. Ok.×12.

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1) Contributions from the Divisions of Plant-Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 264.

a *c* *b*

Fig. 1. *a*, *Papaver lateritium*, (♂); *b*, *P. bracteata*, (♀); *c*, F_1 plant.

Observation

External characters in the F_1 plants. Some external characters in the F_1 plants are intermediate between those of the parent plants, but many characters of *P. lateritium* dominated as shown in Table 1.

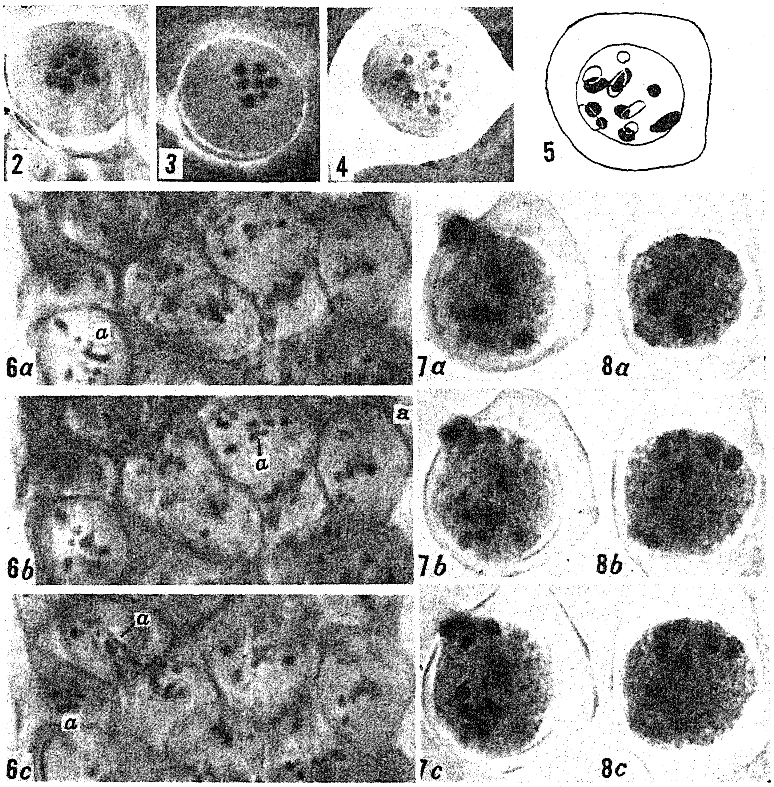
The chromosomes. The somatic chromosome number in the female parent, *P. bracteata*, is 14 (Fig. 2) (cf. YASUI 1937). They form 7 gemini in the meiosis. The male parent, *P. lateritium*, also has 14 somatic chromosomes which form also 7 gemini in the meiosis of the PMCs (Fig. 3) (cf. YASUI 1927, SUGIURA 1940). The behaviour of the chromosomes in the meiosis of PMCs in both parent plants is quite regular and produces healthy pollen grains. The female parent is highly self-sterile, while the male is self-fertile. The somatic chromosome number of the F_1 plants is also 14 which is the sum of the gametic chromosome numbers of the parent plants.

Table 1. External characters in the parents and F_1 plants

Characters	Female parent *P. bracteata*	Male parent *P. lateritium*	F_1 plant
Height of plant	About one meter	About 1/2 meter	About one meter
General appearance	Sturdy	Somewhat slender	Slender
Aerial shoot	Simple, uniflorous	Branched	Branched
Young peduncle	Erect	Nodding	Nodding
Bract	Prominent	Absent	Absent
Colour of petal	Spectrum red	Bittersweet orange	Flame scarlet to genadine
Size of flower	Large	Small	Intermediate
Shape of ovary	Spherical	Obovate	Obovate
Trichomes on floral axis	Stiff, decurrent	Soft, erect	Soft, long, erect; some hairs stiff like those of the female parent
Flower season	Once in spring	Flowering all year round	Flowering all year round
Fertility	Highly self-sterile	Self-fertile	Almost sterile

The chromosome behaviour in meiosis of PMC of F_1 plants. In the early prophase several chromosomes are gathered around a large nucleolus, while later they recede one by one from the nucleolus except 4 large which remain attached until a later prophase just before the nucleolus disappears from the nucleus. Two of the latter have each a small satellite; one of the non-satellited chromosomes is attached at the region of the constriction to the nucleolus and is associated with the distal end of its larger arm with a small chromosome to form a bivalent, and the remaining 4th one also is associated with a small chromosome, which is smaller than the above mentioned, to form another bivalent (Fig. 9). There was observed a bivalent two components of which resembled each other and were associated side by side (Fig. 9, *a*). So that there were 3 bivalents, 2 unequal and one rather homologous one and 8 univalents in the PMC (Figs. 6a-c, 10, 12, 13), but sometimes there were found 5 bivalents and 4 univalents. Consequently we can see that the parent plants have more than 3 pairs of the chromosomes with more or less homologous parts between them. Some of these bivalent components were found associated with each other in the early stage but soon after they separated leaving no sign of the previous association and behaved as if they were univalent chromosomes (Figs. 4 and 5).

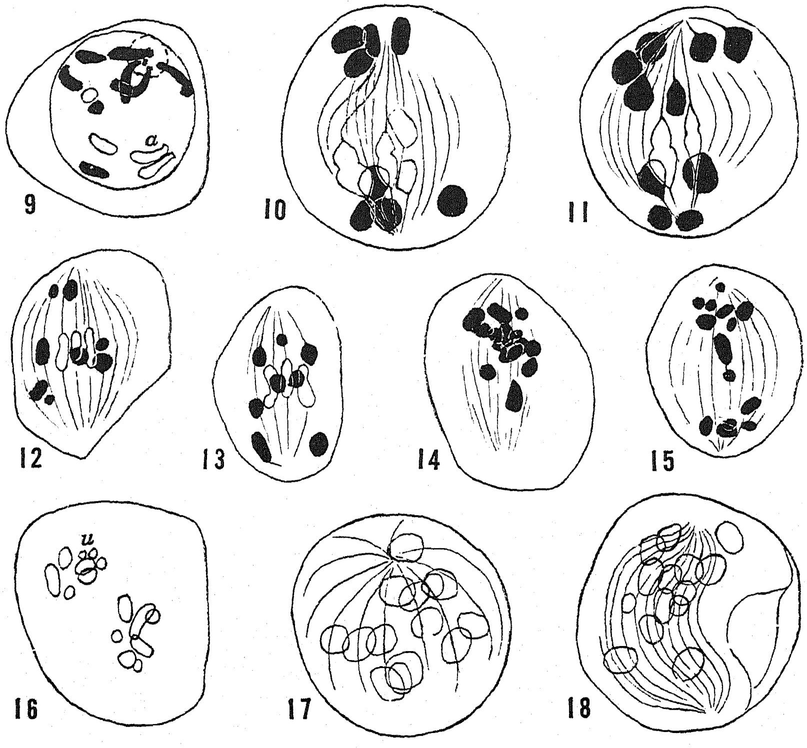
The 3 bivalents mentioned above generally came together into the central region of the equator of the atractosome, and a few univalents, generally less than 4, came into the periphery of the equator (Figs. 12 and 13), while other chromosomes did not enter into the formation of the equatorial plate at all, but were distributed



Figs. 2–8. Photomicrographs of PMCs (×1000), except 5, a camera lucida drawing (×1200). All figures except 6*a*–6*c* are from aceto-carmine smear material; 6*a*–6*c*, NAVASHIN's fixation. 2, polar view of the last metaphase of a PMC of *P. bracteata*; 3, the same of *P. lateritium*; 4, 5, PMCs of F_1 plants, later prophase; 6*a–c*, three optical sections from a paraffin section, *a*, bivalents at the equator; 7*a–c*, a mechanically pressed PMC at different optical levels; 8*a–c*, a PMC at different optical levels, univalents in the upper polar region show traction fibers well.

irregularly in the atractosome and were connected with one pole of the latter with traction fibers and proceeded into the pole earlier than the components of the bivalents (Figs. 6–8, 10–13). The connection between the univalents and the poles with the traction fibers was observed very clearly especially in the aceto-carmine smear preparations (Figs. 7, 8 and 11). A PMC was pressed mechanically during the investigation so that the chromosomes in a

pole (the upper pole in Figs. 7a–c) were pushed out from the cell, but the connection with the traction fibers clearly remained. At that time a bivalent (a bivalent in outline on the left hand side in Fig. 10) was pushed downward, so that the traction fiber from that pole became elongated and curved, as is to be seen also in the figures 7b, c and 10. The bivalent components had separated generally and



Figs. 9–18. Camera drawings of PMCs of F_1 plants. 9, 12–16, NAVASHIN's fixation; 10, 11, 17 and 18 aceto-carmine material. In 10–13 bivalents are shown in outline. 9, later prophase, 2 satellited univalents, 2 bivalents attached to nucleolus, *a*, a bivalent having 2 homologous components associating side by side; **10**, same with 7, *a–c*; 3 bivalents at the equator, one of which is displaced by the mechanical press, having elongated and curved upper traction fibers; 4 univalents in upper polar region are shown in their normal position before being pressed mechanically; **11**, same with 8*a–c*, 2 unequal bivalents at the equator, traction fibers and their connections between univalent chromosomes and a pole; **12**, same with 6*b*, *a*, 3 bivalents and 4 univalents at the equator, 2 univalents near the upper pole, and the remaining 2 proceeding toward the lower pole; **13**, 3 bivalents and 2 univalents at the equator, 3 univalents connected with upper pole 2 at the other polar region, one proceeding to the same pole; **14**, almost all the chromosomes reaching one pole, a few proceeding to other pole; **15**, 5 univalents at lower pole, 6 at upper, one unequal bivalent at equator; **16**, interkinesis, daughter chromosome groups having the chromosomes in the same number, but unequal in sizes; in one group a univalent is divided into two (*u*); **17**, fibers from one pole distributed on the surface region of the atractosome are shown; **18**, a depressed PMC, the atractosome becoming crescent. ×1200.

proceeded into the poles later than some univalents, but often in the form of bivalent.

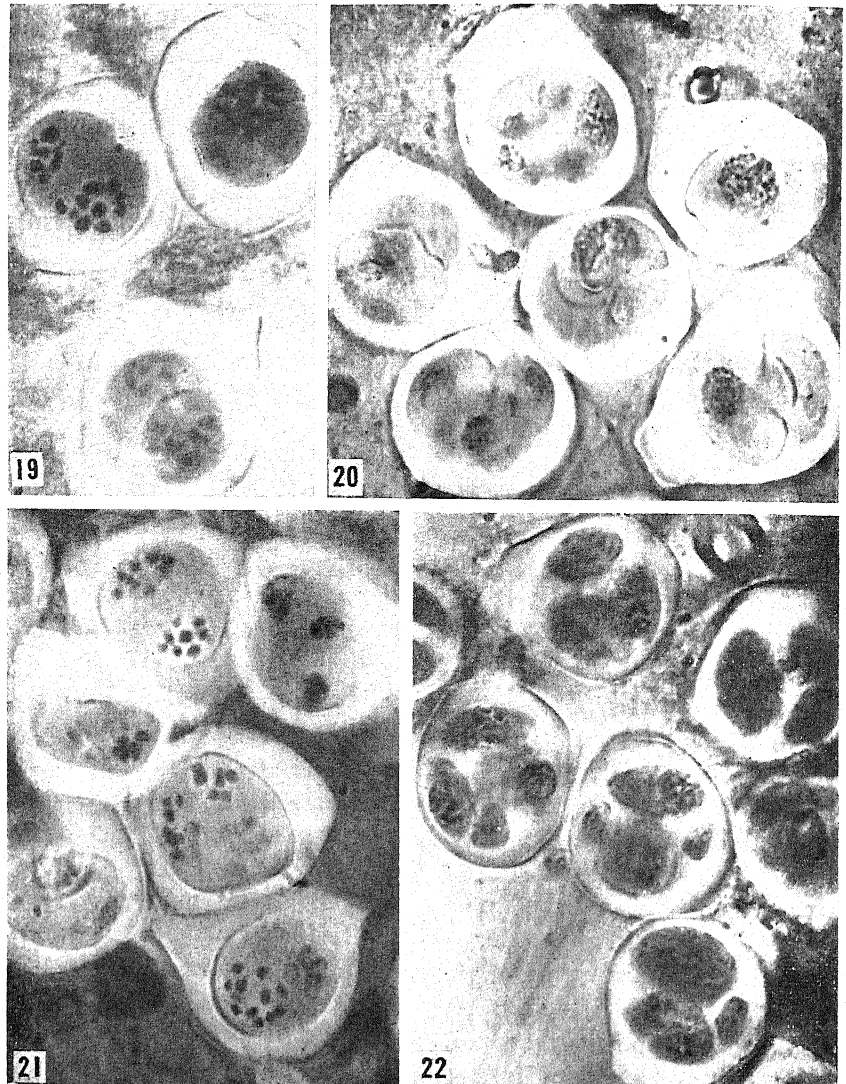
The mode in which the univalents proceeded toward the poles was at random, consequently the chromosome numbers of the daughter nuclei of the 1st meiotic division were different generally, in an extreme case one daughter nucleus having only 3 chromosomes which may be the separated components of the bivalent which formed the equatorial plate, and in another 11. In some nuclei they showed the same number of chromosomes, though even in such cases the sizes of the chromosomes in the nuclei were different (Fig. 16). Scarcely any case of the separation of the daughter chromatids of the univalents in the 1st meiotic division was observed, but they passed into one pole without any actual separation (Fig. 16, *u*).

It was observed in some PMCs that the equatorial region of the cell was depressed, probably due to the rash intrusion of the fixing fluid, and the atractosome was also depressed at the equatorial region, while the poles remained in the original position; consequently the atractosome assumed a crescent shape, the convex side being opposite to the depression (Fig. 17).

In some PMCs fibers radiating from the poles and having no connection with any chromosomes were found, partly belonging probably to the central fibers (Fig. 18). The number of the central fibers was numerous when the fibers were finer, while they were smaller when the fibers were thicker. The difference may be due to an external condition such as the fixing fluid.

After the 1st meiotic division cytokinesis generally occurred in the PMCs, though the divisions were often incomplete and irregular, resulting in the formation of irregular shaped interkinetic cells. Extranuclear chromosomes, miniature nuclei formation, and reconstruction nuclei were observed (Fig. 19).

The second meiotic division was rather regular, but due to the irregularlities of the 1st one the tetrad formation was not regular (Figs. 19–22). Such irregular behaviour, however, occurred in different degrees in the different PMCs in the different individuals as well as in the different flowers of the same plant, and even in the different anthers of one and the same flower. Thus very few fertile pollen grains were produced, so that the formation of healthy seed was rare, even when some good healthy ovaries were pollinated with these pollen grains. I have not examined yet the meiosis in the macrospore mother cells, but probably the same conditions occur in them, because we could also scarcely get seeds even when the F_1 plant was back-crossed with healthy pollen grains of the parent plants.



Figs. 19-22. Photomicrographs of PMCs of the F_1 plants. 19, 3 PMCs of interphase; one having 2, one larger and another smaller, nuclei and an extra-nuclear chromosome, one containing a large reconstructed nucleus and a miniature nucleus, the third in which complete cytokinesis occurred after the 1st division; 20, PMCs in interkinesis and in the 2nd telophase; 21, PMCs in which irregular cytokinesis occurred; 22, PMCs in tetrad formation, almost all of them having miniature grains beside larger grains. ×1000.

Discussion

1. *The homology of chromosomes and syndesis in PMC of F_1 plants.* The chromosomes of the PMCs of *Papaver bracteata* are rather uniform in size, but those of *P. lateritium* are

various; and the chromosomes in the PMCs of F_1 plants show conspicuous differences both in their size and structure. In spite of such differences there are generally more than 3, sometimes 5 pairs of chromosomes found associated to form bivalents in the prophase. At least 2 pairs of these bivalents showed clearly unequality of components. Such unequal associations were reported already in other plants by several investigators (KUWADA 1919, NAVASHIN 1927, 1934, AVERY 1930, ONO and SATÔ 1935 etc.).

Since BELLING and BLAKESLEE (1926) reported the association of the non-homologous chromosomes, the relationship between the homologous parts in the bivalents has been much discussed and was used for the criterion of the affinity between the parent plants. The association of the non-homologous chromosomes in the case of *Datura* does not mean the association of the non-homologous parts of the chromosomes, but the association of the chromosomes of different sets, between the homologous parts of the chromosomes translocated from a chromosome of a different set. Sometimes the association of true non-homologous parts of chromosomes was observed as reported by McCLINTOCK (1933) in *Zea Mays*, but such an association seems to be temporary. Moreover, the recent studies of the salivary chromosomes in *Diptera* show us that the association of chromosomes is really a strong indication of the homology of those associated parts of the chromosomes. The unequal association now in question would seem to give some evidence for the view that the association may occur between different kinds of chromosomes, but the difference in size is not necessarily a sign of the non-homology of the internal constituents of the chromosomes, because 1stly the larger chromosomes may have more heterochromatin than the smaller, though they have the same genic constituents, 2ndly they may have homologous parts between them, namely the smaller one may represent parts of the larger chromosomes, 3rdly they may be homologous in their genic qualities but different in their quantities which may cause the difference in size. These question must be settled by further research.

From the study of the components of the chromosome sets in *Papaver somniferum* and its hybrids with other species, I came to the conclusion that 7 gametic chromosomes in *Papaver* might have been derived from two different ancestors, one having 3 and the other 4 gametic chromosome sets, i.e. *Papaver* having 14 2n-chromosomes are probably amphidiploid (YASUI 1937, 1940). SUGIURA (1940) confirmed the latter view from the study of the chromosome numbers in various sections of this family. Such amphidiploid derived generally from hybrids or their derivatives whose parents

in either cases are rather remote, and the chromosomes in the F_1 plants scarcely associate as we see in the tetraploid *Primula kewensis*, a well known amphildiploid derived by the chromosome duplication from an artificially raised interspecific hybrid whose parents were remote. From this and also the presence of the unequal association of bivalents we can consider that these parent plants, *P. bracteata* and *P. lateritium*, have the gametic chromosome sets in which no autosyndesis could occur. So that the bivalents in the F_1 plants are to be considered as the result of the allosyndesis, in other words *P. bracteata* has more than 3 chromosomes which more or less resemble those chromosomes of *P. lateritium*. To this extent these plants should be allied to each other. Taxonomic relationship after FEDDE (1936) is also in accordance with this conclusion.

2. *Traction fibers and univalent chromosomes.* As described above, there were observed in a PMC most frequently 3 bivalents and 8 univalents, but sometimes 5 bivalents and 4 univalents. In these cases the smaller number of the bivalents was due to the fact of the earlier separation of their association components in an early prophase, and the fact that such separated partners do not show much difference from the true univalents having no partners from the first.

The behaviour of the univalents in the first meiotic division studied by several investigators may be divided into two types, first the case when the univalents divide into two and separate at the equator of the atractosome after the bivalents have passed into the anaphase and followed the preceeding separated bivalent components to the poles (KIHARA in *Triticum* 1919, YASUI in *Papaver* 1921 etc.), secondly the case when the univalents do not divide in the first division and migrate as such into the poles after the bivalent components have passed into the anaphase (ROSENBERG in *Drosera*, 1904 etc.). In these cases the univalents do not move towards the poles before the separation of the bivalent components.

It seems peculier in the present case that many univalents passed into the poles before the bivalent components began to move from the equator to the poles, and also that the movements of the univalents were effected by traction fibers connected with one pole. This peculier behaviour may be due to the earlier differentiation of the attachment points in those univalents and to the fact that most of these univalents are not true univalents but represent the separated bivalent partners.

Simultaneously with the differentiation of the atractosome in the nucleus of the PMC, the chromosomes which were hitherto distributed at the surface region of the nuclear cavity began to move

toward the center of the nucleus. At that stage the attachment points in the bivalent partners were generally differentiated, so that the attachment point of each of them was attached to the free end of the traction fibers streching out from each pole. According to SCHMIDT's consideration (1939) the traction fibers, in which the molecules are arranged lengthwise, are streched out at the beginning of the spindle formation, but the fibers contract in the anaphase to telophase. This process probably resembles that of the contraction of tendrils; the free end of the tendrils stretch out until they come to be attached to something, but as they reach something they begin to contract; traction fibers may also strech out when one end is free, but they will begin to contract when the ends come in contact with the attachment point of the chromosomes owing to some physico-chemical changes occurring in it. At the beginning of the contraction of the fibers, when the tractive forces from both poles and the resistance of the paired chromosomes to those forces are the same and balanced, the bivalents may stay at the equator to form the metaphase. On the contrary those univalents above described which have only one attachment point and offer no resistance as in the separation of the paired chromosomes, and also are subjected to no tractive force from the other pole, may proceed toward the pole earlier than the bivalents.

We cannot agree with BĚLAŘ's view (1929) of "Stemmkörper" which is considered to develop between the daughter- or separated chromosome groups and push them toward the poles. Because we see that the polar movement of the univalents here described has not been effected by the aid of such a body as "Stemmkörper", but they were pulled only by the traction fibers.

Summary

1. In the F_1 plants of *P. bracteata* × *P. lateritium* some characters were intermediate between those of the parent plants, but many other characters of the male parent, *lateritium*, dominated over those of the female parent, *bracteata*.

2. The chromosome number of the parent plants was the same, but their sizes were different; the chromosomes in *lateritium* being generally smaller than those of *bracteata*. The chromosome number in the F_1 plants was $2n = 14$, just the sum of the gametic chromosome number in the parent plants. The variation in chromosome sizes was greater than either of those of the parent plants.

3. In the meiosis of the F_1 plants there were found more than 3 pairs, sometimes 5 pairs of gemini, some of them being unequal

and others separating early in the later prophase and behaving like univalents. Many univalents, probably the just mentioned, proceeded earlier than the bivalents into the poles, though their distribution was at random as we see usually in the non-splitting univalents in the 1st meiotic division. Generally 3, sometimes 2, bivalents formed an equatorial plate after many univalents had gone into the poles, and followed them after separation or without separation of partners. Simultaneously when the bivalents were at the equator few univalents were found in the periphery of the equatorial region; the latter has passed into the poles without splitting.

4. The cytokinesis generally occurred after the 1st meiotic division, though often it was incomplete. Several irregularities in the division were observed. Second division was rather regular, but due to the irregular behaviours in the 1st division very few good pollen grains were produced. The F_1 plants were highly sterile even when they were back-crossed with healthy pollen grains of the parent plants.

5. Traction fibers were observed clearly especially in the aceto-carmine smear materials. The univalents were connected with one pole by the traction fibers which may play the role of the movement of the univalents without aid from any other additional forces, e.g. the "Stemmkörper", which was considered by BĚLAŘ as a separator of the daughter chromosome groups toward the poles.

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Karyological Studies in *Saccharomyces cerevisiae*[1) 2)]

By

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(With 34 figures)

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The nucleus of the yeasts has long since been a subject of karyological investigations, yet a number of problems still remain to be settled, i.e. as regards 1) the structure of the nucleus, 2) the number of chromosomes, 3) the mode of division, mitosis or amitosis, and 4) meiosis. The present paper deals with the results of investigations concerning the former three problems.

Material and Methods

As the plant material a strain of *Saccharomyces cerevisiae* was used. Various methods of investigation were employed: smear preparations treated with a drop of acetocarmine and after a few minutes covered with a cover glass; those fixed with Kaiser solution for a few minutes, washed and then treated with acetocarmine; those fixed with Flemming solution, washed, bleached with H_2O_2 and then treated with iron acetocarmine; those fixed with either Kaiser, Bouin, Bouin-Allen, Navashin solutions or chromacetic acid, washed and then stained with Heidenhain iron alum haematoxylin; those fixed with various fixations, hydrolysed in n HCl at 60°C for a few minutes and stained either according to the Feulgen method or with Heidenhain haematoxylin, the latter being sometimes counterstained light green.

To remove various kinds of granules in the cytosome (cell body) the plant was either hunger cultured in distilled water or else its dried preparation was treated with a 1% suspension of either takadiastase, pepsine or trypsine for 2–26 hours, then fixed either with Navashin solution or by heat and finally stained with Heidenhain haematoxylin. Sometimes dried smear preparations were treated with a mixed suspension of the three ferments above mentioned in the proportion of 1:1:1 for 2–26 hours and then stained with Heidenhain haematoxylin. A portion of the colony was fixed with either Navashin or Flemming solution, sectioned according

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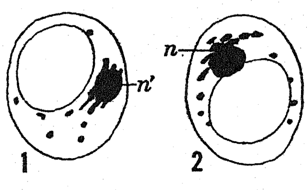
to the ordinary paraffin method and stained with Heidenhain haematoxylin.

In order to get polyploid nuclei the yeast cells were cultured in 0.1, 0.01, 0.05, 0.005, or 0.001% aqueous solutions of colchicine, after which a small amount of cane sugar was added and the whole left for 24, 48 or 96 hours. The cells thus cultured were smeared on a slide, fixed with Kaiser solution and then stained with Heidenhain haematoxylin. A part of the cells were either left in the colchicine solutions for 24 or 48 hours or cultured in turnip extract, after which a small amount of cane sugar was added and the whole left for 24 hours. Such cells were fixed with Kaiser solution and then stained with Heidenhain haematoxylin. The yeast cells were also stained en masse with various solutions of aniline dyes for observation purposes.

Results of the Observations

Though the observation on the living cells was very difficult owing to the presence of many granules, yet a body corresponding to the nucleus seen in fixed and stained materials could sometimes be found near the central large vacuole. In the unstained preparation fixed with various fixatives a nucleus could also sometimes be seen near the central vacuole and besides a dumbbell shaped nucleus was observed at the constricted portion or isthmus between the bud and the mother cell. As will be stated later the dumbbell shaped nucleus is one of the daughter nuclei, which is in the stage of travelling from the mother cell into the bud, and not one that is dividing amitotically.

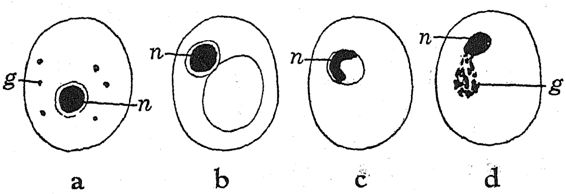
In either the living cells or in the fixed, unstained cells the nucleus showed a homogeneous structure and was reflingent, but was discerned with difficulty from other granular bodies. When stained with various solutions of aniline dyes the nucleus showed up in the same tone as the other granules. When stained with haematoxylin after fixation with various fixatives the nucleus was also stained in a shade similar to that of the other granules, but it becomes, either alone or together with a number of granules, distinguishable when the differentiation was adequately carried out. When the cells were fixed with either Kaiser, Bouin, Bouin-Allen or Navashin solution the granules around the nucleus became so distinct as to prevent the



Figs. 1–2. 1, cell in which a nucleus is not clearly seen. *n'* the portion where the nucleus is thought to be present. Bouin-Allen. ×540. 2, cell showing a nucleus (*n*) clearly. Bouin-Allen. ×5400

accurate observation of the nucleus (fig. 1), though in some cases it was clearly recognized (fig. 2).

In the cells fixed with Flemming solution, the bleaching agent being H_2O_2, the nucleus was distinctly observed only when the differentiation was sufficiently carried out (fig. 3). The nucleus contains a large karyosome around which there is a thin hyaline zone surrounded by a nuclear membrane which is very faintly stained. The karyosome is generally spherical, but sometimes it takes on an irregular form (fig. 3c). Small granules assembling around the nucleus often apparently make it either granular in its structure or irregular in its form. When the nucleus and the central large vacuole lie one upon the other the former may look like a nucleolus and the latter like a nuclear body, and this seems to have led certain earlier observers to err in recognizing the yeast nuclei. Sometimes the karyosome was so large that the hyaline zone around it could hardly be observed, while the size of the nucleus showed a considerable variation in different cells. When the cells were fixed with Flemming solution the granules around the nucleus were often separated en masse from the latter giving a dumbbell shaped appearance which may simulate an amitotic form of behavior of the nucleus (fig. 3 d).



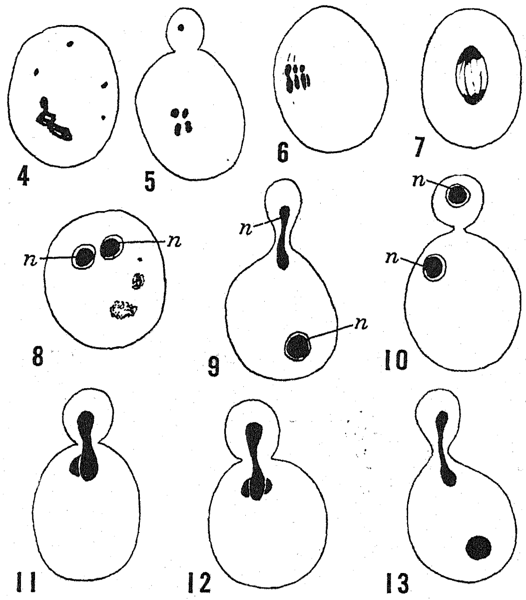
Figs. 3. Cells showing nuclei clearly. Flemming. *n*, nucleus. *g*, granule. ×5400

In order to dissolve off some of the contents of the cell, a 1% suspension of either takadiastase, pepsine or trypsine was applied. By this means the nucleus in some cases was made sufficiently clear for observation, but in other cases the granules were deformed and so prevented observation of the nucleus. Sometimes the nucleus showed a granular structure which was probably the result of dissolution of a part of the nuclear substance by the ferments above mentioned (figs. 26, 27). In some cases irregular rod-shaped bodies appeared (figs. 28, 29).

When a drop of iron acetocarmine is added to the living or fixed cells, left for a few minutes at room temperature, the nucleus stains somewhat deeply and is thus rendered distinguishable from the other stained granules.

Based on the data obtained from the observation on the material either fixed with Flemming solution and stained with haematoxylin

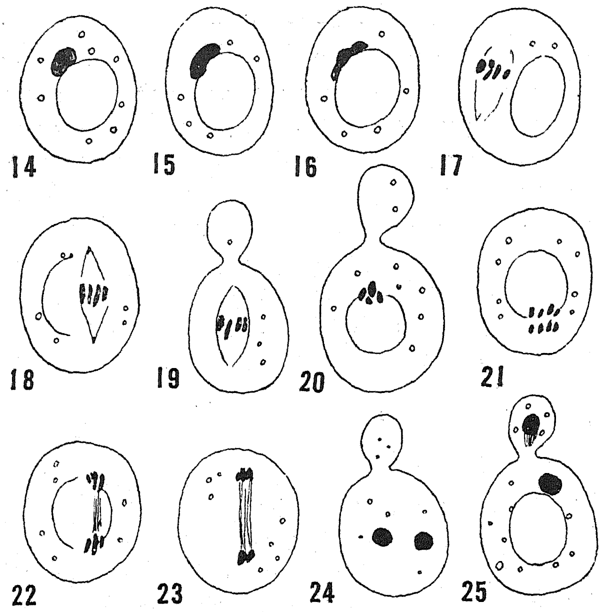
or treated with iron acetocarmine, the process of mitosis will be described (cf. figs. 4–25). When the nucleus enters into the stage of division it gradually shows an irregular form and comes to show entangled chromatic threads (figs. 4, 14–16) which are transformed in turn into four rod shaped chromosomes (fig. 5), while an atractosome (spindle) is formed (figs. 17–19). Fibers are often seen between the two poles of the spindle (fig. 6). Each chromosome divides, perhaps longitudinally, and the resulting halves go to the opposite poles (figs. 6–7, 21–23) to form two daughter nuclei (figs. 8, 24). A telophase figure was often observed (fig. 7). Mitosis occurs either shortly before, during or after budding.



Figs. 4–13. 4–10, showing mitotic nuclear divisions. Flemming. n daughter nucleus. ×5400. 11–13, stages in which one of the daughter nuclei is entering into the bud and another one is found either near or apart from the travelling nucleus. Acetocarmine. ×5400

One of the newly formed nuclei showing a dumbbell shaped appearance, passes into the bud, while another one remains in the mother cell (figs. 9, 11–13). As a result the bud and the mother cell each come to contain one nucleus (fig. 10, 25). After this process the bud grows and is separated from the mother cell by a constriction. While one of the daughter nuclei, also dumbbell shaped, travels into the bud, another one is found either near the posterior portion of the former or apart from it in the mother cell (figs. 11–13). When the remaining nucleus, however, is hidden behind the posterior portion of the dumbbell shaped one it may escape one's attention so that it appears as if only the dumbbell shaped nucleus is present in the cell (figs. 11, 12).

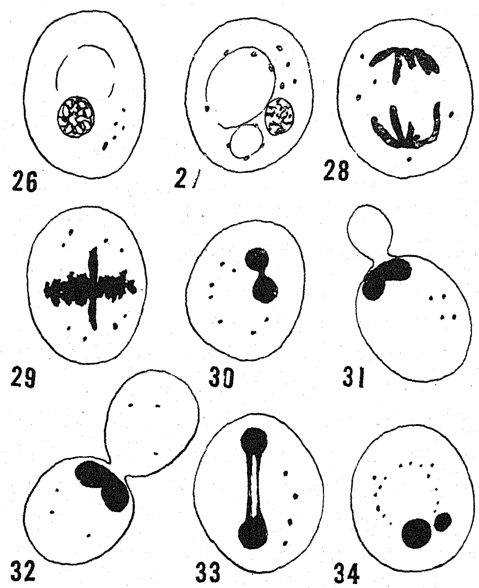
By the Feulgen nucleal staining method the nuclei stain faintly reddish violet, and sometimes the small granules around it show a similar color. All the portions of the cell, with the exception of the nucleus together with some granules, remain unstained. The dead



Figs. 14–25. Showing mitotic nuclear divisions. Iron acetocarmine. ×5400

cells are all stained reddish violet in almost every case.

The cells were immersed in a 0.1, 0.05 or 0.01% aqueous solution of colchicine, after which a small amount of cane sugar was added and the whole left for 24 or 48 hours, fixed with Kaiser solution and then stained with Heidenhain haematoxylin. In these cells neither abnormal nuclei nor irregular mitoses were observed. After 96 hours, however, the cells which contain a large nucleus were found amongst the normal ones. Such abnormal cells were not large in size. The large nuclei may be restitution or polyploid ones induced by the action of colchicine, the atractosome being disturbed to form. In some cells the large nuclei, which were sometimes dumbbell shaped (fig. 30), were often stopped at the outlet of the isthmus, their long axis being parpendicular to that of the latter (figs. 31, 32).



Figs. 26–34. 26, 27, cells stained with haematoxylin after treatment with takadiastase. ×5400. 28, 29, irregular stainable bodies produced by the takadiastase treatment. ×5400. 30–32, dumbbell shaped nuclei induced by the colchicine treatment. ×5400. 33, 34. abnormal mitoses. Colchicine treatment. ×5400

In the cells which were immersed in 0.1, 0.05 or 0.01% aqueous solutions of colchicine, followed by addition of a small amount of cane sugar,

and which 24 hours afterwards were cultured in turnip extract for 48 hours, abnormal nuclei were often found. In some cases the daughter nuclei were connected with each other by a thin thread (fig. 33), while in others both large and small nuclei were found in a single cell (fig. 34). Large or probably restitution nuclei were also often observed. After 72 hours irregular mitoses were not found, but cells with a large nucleus were also often found, which occurrence seems to be a result of the effect of colchicine. In such cells a study of the chromosomes has not yet been carried out. In the culture of the cells with large nuclei it was found that they did not form large progeny cells, though their nuclei were expected to be polyploid.

Discussion

Wager (1898) found that all yeast cells contain a nucleus which consists of a nucleolus and a nuclear vacuole, and later with Peniston (1910) confirmed and extended his findings. The results of other investigators, however, proved that the nuclear vacuole and the nucleolus of Wager were in fact the central large vacuole and nucleus respectively. Feinberg (1902) believed from the staining reactions that the nucleus ('Kernpunkt') of yeast is composed of chromatin substance but not of nucleolus or nucleolar substance. The same observer could not find evidence of the nuclear network, while according to Marpmann (1902) the nucleus of yeast could be stained with either haematoxylin, fuchsin or gentian violet. Fuhrmann (1906) stated in connection with the study of *Saccharomyces ellipsoideus* I that the nucleus is an assemblage of chromatin granules around which a 'Kernhof' is observed. Achromatic spindle and perhaps centrosome are also formed. According to Guilliermond (1910) in *Saccharomyces cerevisiae* and *S. ellipsoideus* the nucleus is composed of an outer nuclear membrane and an inner hyaloplasm in which a central nucleolus and network are observed. A similar structure was recognized by Kater (1927) who opined that the nucleus is made up of the outer membrane, the network and the central nucleolus or karyosome.

These authors all recognized the presence of a nucleus in yeast and especially Guilliermond and Kater showed morphologically that it was similar to that of the higher plants. The present writers also clearly observed this nucleus which stains reddish violet by the Feulgen process (cf. Beams et alii 1940) and undergoes mitosis, as in the higher plants. The nucleus is composed of the membrane, the thin hyaline portion and the central large karyosome. The network, however, was not observed clearly.

Concerning the mode of nuclear division there are two alternatives hitherto advanced. Wager (1898), Guilliermond (1910) and more recently Beams, Zell and Sulkin (1940) insisted on the occurrence of amitosis, while Swellengrebel (1905), Fuhrmann (1906), Kater (1927) and Badian (1937) considered that mitosis occurred. According to Wager (1898) and Wager and Peniston (1910), "at first the young bud contains protoplasm only, but as development proceeds the nuclear vacuole begins to pass into it. Then the nucleolus [nucleus] makes its way to the base of the opening of the mother-cell into the bud and at once begins to divide". Guilliermond (1904, 1910, 1919) observed too in *Saccharomyces cerevisiae* and *S. ellipsoideus* that the nucleus divides also amitotically at the constricted portion between the bud and the mother cell. Recently Beams, Zell and Sulkin (1940), using modern technical methods, observed and described the amitotic nuclear division in *Saccharomyces cerevisiae.*

On the other hand Fuhrmann (1906), confirming the work of Hirschbruch (1902), observed in *Saccharomyces ellipsoideus* I that the nucleus divides mitotically and that one of the daughter nuclei enters into the bud. He also stated that, as the nuclear division proceeds, the nuclear membrane disappears and 4 chromosomes appear in the spindle. Kater (1927) showed in *Saccharomyces cerevisiae* that the nucleus undergoes mitosis and that an undetermined number of chromosomes apparently form, divide, separate and give rise to daughter nuclei in much the same way as in *Phaseolus.* According to him the linin strands connecting the nucleolus and the nuclear membrane probably represent sheaths of chromosome vesicles. Badian (1937) reported mitosis in both *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae,* which have 2 chromosomes in their primitive nuclei and 4 chromosomes in zygotes.

The present writers also recognized mitosis in the vegetative cells of *Saccharomyces cerevisiae* and found the chromosome number to be 4. The nuclear division resembles that of the higher plants. The resting nucleus gradually changes into thread forms which are transformed into chromosomes. At this time the nuclear membrane disappears and the atractosome (spindle) is formed in which the chromosomes divide to form two daughter nuclei. Beams, Zell and Sulkin (1940), confirming the work of Richards (1938) who said "that colchicine does not inhibit the budding process in yeast", concluded that "[this] seems to lend strong support to the view that the budding mechanism does not take place by means of a mitotic process". In the present experiment with colchicine, however, the formation of atractosome was disturbed and abnormalities of the

chromosome mechanism were induced as seen in the case of higher organisms.

One of the newly formed daughter nuclei, according to the present writers, assumes a dumbbell shape and migrates into the bud cell. This behavior of the travelling nucleus closely resembles that of the nuclei in certain kinds of Basidiomycetes fungi in which every one of the four nuclei produced by meiosis enters into each of the basidiophores, taking on the same dumbbell shape. Therefore the dumbbell shaped nucleus which has hitherto been thought to be the amitotic figure is nothing but the stage in which one of the daughter nuclei is travelling through the isthmus. The other daughter nucleus remains in the mother cell either near or apart from the posterior portion of the dumbbell shaped one. When the nucleus of the mother cell is quite near to, or hidden behind, the posterior portion of the dumbbell shaped nucleus it may escape the attention of the observer.

In the figures of certain previous authors we also often find two nuclei in one cell, even in the cell in a non-budding condition, but they shed no light on the present case in which the two nuclei, one of which entered into the bud, were the product of mitosis.

Summary

1. The nucleus of the vegetative cells of *Saccharomyces cerevisiae* is composed of a central deeply stained large karyosome, with a narrow hyaline zone around it, and an external membrane. The form of the karyosome is generally spherical, but sometimes it is irregular. It sometimes showed a granular structure in the case of treatment with ferments such as takadiastase. Small granules are often found around the nucleus.

2. The nucleus and also some granules are stained by the Feulgen nucleal reaction.

3. The nucleus undergoes mitosis. The resting nucleus becomes transformed into thick threads which become 4 chromosomes. The latter divide in the atractosome (spindle) to form two daughter nuclei.

4. After mitosis one of the newly formed daughter nuclei, taking on a dumbbell shape, travels through the isthmus into the bud, while the other one remains in the mother cell as its nucleus.

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Studies of Mitosis and Meiosis in Comparison
III. Behaviour of chromonemata in the pre-leptotene stage in meiosis

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The beginning of mitosis is characterized by the occurrence of the spiral stage. This stage has been described by some authors as occurring also in meiosis, and in animals there are known to exist some variations in the behaviour of chromonemata in this beginning stage of meiosis (WILSON, 1925; cf. KUWADA, 1939). In the comparative study of mitosis and meiosis, it is very important to ascertain clearly how the chromonemata behave in the beginning stage of division in both mitosis and meiosis, and to know how the beginning of meiosis differs from mitosis. In the present paper, the results obtained in the study of the early prophase of meiosis in *Tradescantia reflexa*, *Trillium* sp., *Vicia Faba*, *Psilotum* sp. and *Fritillaria* sp. are reported.

Method

In the cases of *Trillium* and *Fritillaria* using the anthers of relatively large sizes, the contents of the anther were pressed on the slide out of a cut made at one end with a sharp knife, and were fixed with various fixatives such as FLEMMING's or CARNOY's mixture for a moment, fixation being followed immediately by the staining with acetocarmine. Smaller anthers and sporangia of *Vicia*, *Tradescantia* and *Psilotum* were, after being immersed in toto for a moment in one of the fixatives, crushed gently on the slide by means of a pincette, and then stained with acetocarmine. In the cases of *Trillium*, *Tradescantia* and *Fritillaria*, paraffin sections prepared by the usual method were also used for comparison.

The variations in size and colour of the anthers and sporangia serve, in some measure, for arranging them in due order according to the stages of meiosis. Internal characteristics also serve for the same purpose. In the sporogenous cell complex the nuclear divisions show every stage of mitosis. In the advanced stage toward meiosis, however, it becomes noticeable that there is a tendency of the complex to have all its cells uniformly of the same stage. Most

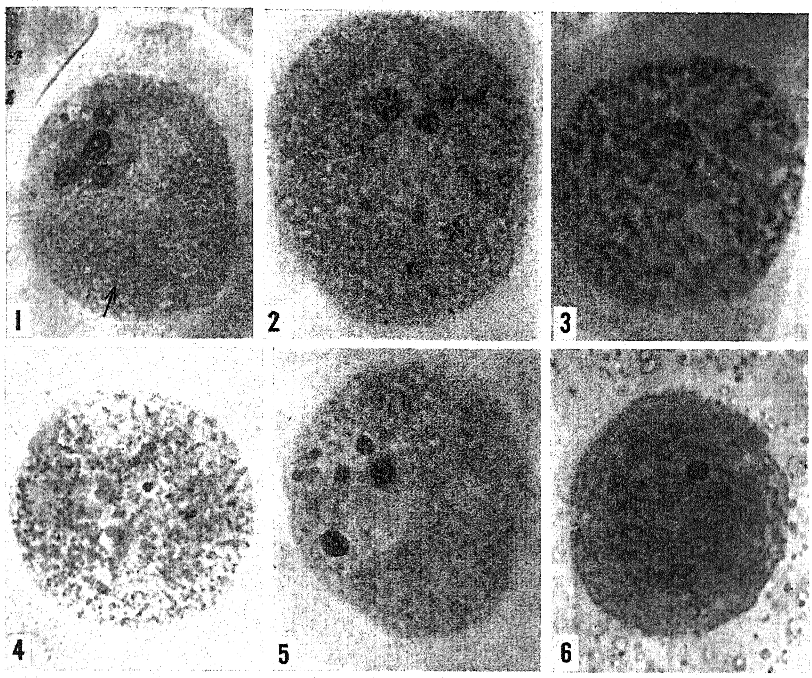
cells are found at telophase or interphase in this stage, and in the final premeiotic stage all of them are at interphase. In these stages, the cells are closely associated with one another, so that they are polygonal in shape. As the compact association of the cells becomes gradually loosened, the cells are rendered spherical. The leptotene stage is reached when these cells are undergoing this transformation of the cell shape from the polygonal to the spherical. In the case of *Psilotum*, there is another peculiarity showing at what stage of development the cells are, namely the mode of the grouping of the cells which becomes noticeable when the contents of the sporangium are pressed out by a pressure given on the cover glass. In the stage where the divisions are premeiotic or in the interphase which immediately preceds the meiosis, the grouping is irregular, and it becomes regular sooner or later thereafter. In the earliest stage of the regular grouping, the number of the cells in a group is found to be 32. It is then reduced to 16, 8 and 4, as the stage advances. When the number is 4, the cells are mostly at the leptotene stage. Slight changes in stage can be followed from one to another in due order, if the cells are found in the same loculus, on the assumption that they are nearly of the same stage. The size of the cell is not a reliable characteristic for the determination of the stage, because it may be changed by a slight pressure given on the cover glass. Also no remarkable cytoplasmic contents which can serve for the purpose of the stage determination are found before leptotene.

Observation

Fritillaria was studied only fragmentally. In the other plants which were studied more closely, the results obtained were mainly the same.

Last premeiotic interphase. In the interphase just preceding the meiosis, the nucleus appears to be finer in structure, the chromonema distribution being more uniform, than in the preceding telophase (Figs. 20 and 21). The region of the nucleoli appears to be hollow without showing any visible structure. The fine convoluted chromatic threads, with which the nucleus is filled, form a complex in which they are hardly traceable along their length, but in some favourable cases more or less coiled threads can be traced through some length especially in the cases of *Trillium* and *Psilotum* (Figs. 1 and 7, the regions indicated by arrows). In *Trillium*, several large heteropyknotic bodies (chromocenters) are found, which tend to gather at a certain region of the nucleus (Fig. 1) or to lie sur-

rounding the region of the nucleolus (Fig. 2). Besides these chromocenters, many small similar bodies are also found scattered in the nucleus. The parallel orientation of the zig-zag or convoluted threads or the telophasic orientation of the chromosomes is occasionally noticeable. In *Vicia* and *Fritillaria,* chromocenters are smaller



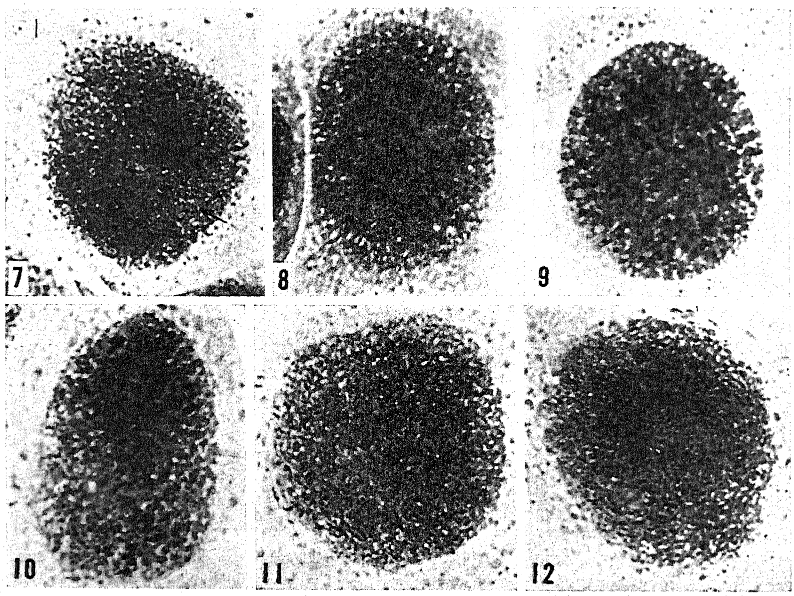
Figs. 1–6. *Trillium* sp. Zeiss' homog. imm. 1/12×oc. 4. 1. Interphasic nucleus immediately preceding meiosis, showing uniform nuclear structure and some heteropyknotic bodies gathering in group. 2. Beginning of meiosis, showing the disturbance in uniformity of the nuclear structure taking place. Some number of coiled threads standing out clearly from the others are visible. The heteropyknotic bodies are found lying around the nucleolus. 3. "Spiral stage" or "contraction stage" with contracted chromosomes showing spiral structure. 4. Early stage of unravelling. Spirals of individual chromosomes are still distinguishable to some extent from those of the others. 5. Later stage. The spirals are almost completely drawn out. 6. Leptotene stage with straightened out chromonema threads. One heteropyknotic body is shown.

than those of *Trillium* and show no marked tendency of gathering together (Fig. 13). In *Psilotum* and *Tradescantia,* no such bodies as chromocenters are found in the nucleus (Figs. 7 and 21).

Meiotic prophase. The noticeable fact in the beginning of meiosis is that the nuclear structure is coarser in general appearance than in the preceding interphase (Figs. 2, 8, 14 and 22). The chromatic threads seem rather thicker (Figs. 14 and 22). In certain regions of the nucleus their coiled aspect is more conspicuous

than in the preceding stage (Fig. 2), and a disturbance takes place in the uniform distribution of chromonemata (cf. KUWADA, SINKE and NAKAZAWA, 1939), the interchromosomal spaces becoming more clearly perceptible.

Next it becomes clear that a number of coiled threads or chromosomes stand out clearly from one another leaving clear spaces between them as shown in Figs. 3, 9, 15, 19, 23 and 24. This stage nearly corresponds to the so-called spiral stage in mitosis and is characterized by the contraction or condensation of the chromosomes

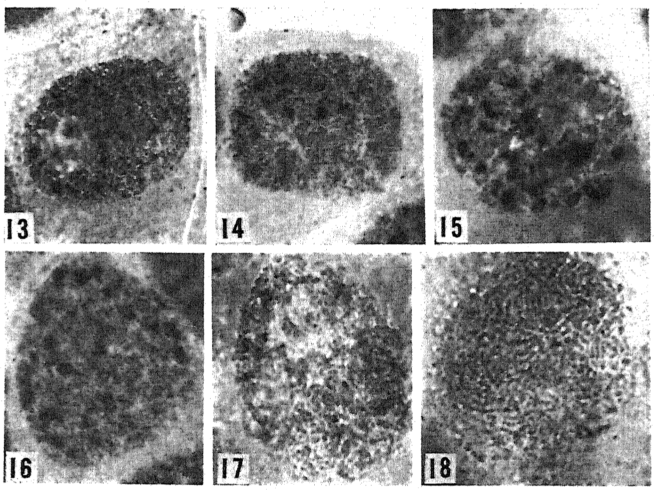


Figs. 7-12. *Psilotum* sp. Zeiss' 1/12×oc. 4. 7. The last premeiotic interphase. 8. Beginning of meiosis. 9. "Spiral" or "contraction stage". 10. Early stage of unravelling. Unravelling is taking place in the lower region of the nucleus. 11. Later stage. 12. Leptotene.

from their diffuse state in the preceding stage, being comparable with the prochromosome stage in animals (WILSON, 1925). In *Trillium* and *Vicia*, the spirals or condensed chromosomes run irregularly in the nucleus, but can be traced through a considerable length (Figs. 3 and 19). In *Tradescantia* and *Psilotum*, the condensed chromosomes are highly rich in chromatin, and the condensed aspect is more conspicuous than in the former two plants (Figs. 9, 23 and 24). In *Fritillaria*, the condensation goes so far that there appear in the nucleus many irregular compact masses which remind us of the chromocenters (Fig. 15). It seems probable that according to the species of plants the condensation takes place in varying

degrees, as has been described in the case of the animal prochromosomes (WILSON, 1925).

If the condensed masses or the strands of condensed coiled threads really correspond with the chromosomes, they should be found in the diploid number. In *Trillium* and *Vicia*, the free ends of these strands were often observable, and an attempt was made to count them, but the difficulty of tracing the strands through their whole length made it impossible to get a conclusive result. From the comparison, however, in various morphological features of the coiled threads or spirals with those in the premeiotic telophase where they represent single chromosomes, it seems highly probable that these spirals must correspond each with a chromosome. There is, indeed, a more or less remarkable resemblance also in the general appearance between the nuclei in this stage and those in the telophase



Figs. 13–18. *Fritillaria* sp. Zeiss' 1/12×oc. 4. **13.** Nucleus in the last premeiotic interphase with small heteropyknotic bodies. **14.** Beginning of meiosis. **15.** "Spiral stage" or "contraction stage". Condensed bodies, in appearance like chromocenters, are seen. **16.** Early stage of unravelling. In the condensed bodies spirality is visible. **17.** Later stage. **18.** Leptotene stage. No heteropyknotic body is found.

of the preceding division, but the general straight arrangement of the chromosomes is much less conspicuous in the former nuclei than in the latter. In mitosis, if the cell is of elongated shape, the general straight arrangement is very conspicuous indicating that in this case the telophasic chromosome arrangement is largely maintained. The spherical shape of the nucleus in the meiotic prophase seems responsible for this inconspicuousness of the general straight ar-

rangement of the chromosomes in this stage in meiosis (cf. KUWADA, SINKE and NAKAZAWA, 1939).

The stage of unravelling then follows. The spirals become first loosened (Figs. 16 and 25), and are then drawn out (Figs. 4, 10 and 25). The chromocenter-like bodies found in *Fritillaria* now come to show their spirality (Fig. 16). The unravelling often commences at a certain region of the nucleus (Figs. 10 and 25), and the territorial or aggregated structure of the nucleus in the spiral stage becomes more and more obscure (Figs. 5, 11 and 17). The drawn out threads do not show any marked change in their thickness. This is a difference between the cases of meiosis and mitosis, because in the latter case a remarkable increase in thickness of the threads is recognizable (cf. KUWADA and NAKAMURA, 1934). The fully drawn out threads fill up the nucleus evenly. This is the leptotene nucleus (Figs. 6, 12, 18 and 26)[1].

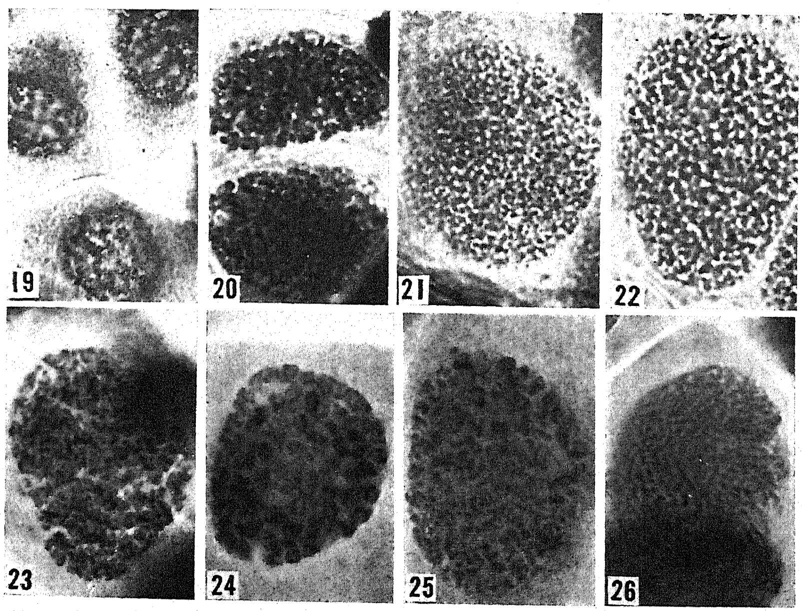


Fig. 19. *Vicia Faba*. Zeiss' 1/12×oc. 4. Three nuclei in the "spiral stage". Coiled threads of condensed form are seen in each nucleus. Figs. 20-26. *Tradescantia reflexa*. Zeiss' 1/12×K 8. 20. Two telophasic nuclei in the last premeiotic mitosis. 21. A nucleus probably in the last premeiotic interphase. 22. Nucleus in later stage. 23. Early "spiral stage" or "contraction stage", showing the general straight arrangement of the chromosomes. 24. "Spiral stage" in maximum chromosome contraction. 25. Unravelling stage. In the lower region of the nucleus, unravelling is seen. 26. Leptotene stage.

In *Trillium*, the heteropyknotic bodies exist as solid bodies throughout the stages before leptotene (Figs. 1, 2, 3, 4 and 5) and

1) In advanced stages peculiar arrangements of the threads in "whirl," "bouquet" and "asters" are observed in *Tradescantia, Psilotum* and *Trillium*.

also even in leptotene (Fig. 6) and pachytene. In *Vicia* several such bodies are also found in the leptotene nucleus. In *Fritillaria*, the heteropyknotic bodies observed in the interphase are no longer found in the leptotene, and the nucleus shows in this stage only delicate leptotene threads as in *Tradescantia* and *Psilotum* (Figs. 12, 18 and 26). It seems highly probable that the behaviour of the heteropyknotic bodies in the meiotic prophase is different in different plants.

Conclusion

The "Ansammlung der Chromatinkörnchen zu Klümpchen" described in some higher plants in the early days of investigation as a phenomenon taking place at the onset of meiosis probably corresponds with the chromosome condensation. MIYAKE (1905) says that "Wenn der Kern beinahe ausgewachsen ist, fangen die Chromatinkörner an, sich an verschiedenen Stellen zu sammeln. Die Zahl der Sammelpunkte scheint in manchen von mir untersuchten Pflanzen ungefähr der normalen Zahl der Chromosomen zu entsprechen". OVERTON (1905) describes: "Bei *Thalictrum purpurascens* verwischen sich die Umrisse der einzelnen Körnchen, nach und nach sind alle vereinigt zu einem größeren Körper, der sehr viel länger als breit ist und so in der Form an ein Chromosom erinnert". Since then, many authors have observed that the preleptotene threads with the zig-zag or spiral aspect come out from the so-called reticulum (BONNEVIE in *Allium*, 1911; CHIPMANN in *Lilium*, 1925; NEWTON in *Tulipa*, 1926; SZAKIEN in *Osmunda*, 1927; TAYLOR in *Gasteria*, 1931; HORTON in *Triticum*, 1936 and others). BONNEVIE describes it thus: "Unter Auflösung der zwischen ihnen befindlichen Anastomosen treten die auf dem Chromatinknoten befestigten Fädchen allmählich in ganzer Länge zum Vorschein, indem sie immer noch zickzackförmig gebogen oder spiralig gedreht erscheinen". NEWTON states that "At the end of the resting period, however, the formation of zigzag threads, very similar to those of the ordinary prophase, precedes the lengthening out into the leptotene threads which are impossible to count, though free ends can occasionally be seen". According to SINKE (1934), who studied this stage with special attention, the coiled preleptotene threads are "quite free from their neighbours". NEBEL and RUTTLE (1936) have called this stage the "premeiotic spiral prophase". They, comparing this stage with the somatic spiral prophase, have come to the conclusion that "The premeiotic spiral prophase is the first stage in meiosis which is distinct from mitosis in that at this stage the threads become thinner, less chromatic, and more elongate than in the corresponding somatic

spiral prophase". All these can be regarded as showing, in accordance with the result we obtained, that the leptotene thread formation is preceded by the condensation of the chromosomes of spiral structure.

While in animal meiosis, some different types have been described in the formation of the leptotene threads (WILSON, 1925), in plant meiosis it is generally assumed that the leptotene threads directly come out of the nuclear reticulum, without emphasis being laid on the fact of the chromosome condensation (SHARP, 1934; GEITLER, 1934). The first visible change in meiotic division is, however, as mentioned above, not a simple drawing out into thin threads of the coiled chromomenata which formed the reticulum, but a condensation of the chromosomes which formed the "reticulum" in their diffuse state, or in other words, the restoration of the telophasic condition of the chromosomes. The drawing out is a phenomenon which follows this condensation. The chromosome condensation is a characteristic feature in the very beginning of meiosis as well as in mitosis, and we are led to the conclusion that NEBEL and RUTTLE's statement that "Any account of meiosis commencing with leptotene is thus incomplete", is reasonable. There is a considerable difference in the degree of condensation as seen between *Trillium* and *Fritillaria.* After condensation, unravelling and elongation of the threads take place thus resulting in the formation of the delicate leptotene threads.

Comparing the form change of the chromosomes mentioned above with the animal prochromosome formation, we come to the inference that in plants also there may be some modifications in the mode of the leptotene thread formation, especially in respect to the difference in degree of chromosome condensation. It is necessary to carry out further comparative studies of early meiotic prophase in various plants, especially with regard to the behaviour of heteropyknotic bodies. The tentative conclusion which we can draw at present may be as follows: the behaviour of the chromonemata in the beginning of the meiotic division is fundamentally the same as that observed in the somatic early prophase, except in the mechanism of the chromosome unravelling which demands further investigation.

Summary

The main results obtained in the present investigation may be summarised as follows:

1) In the last premeiotic interphase the nucleus is of the chromonema structure with uniform distribution.

2) The first sign of the visible change in nuclear structure in

the meiotic prophase is a disturbance in uniformity of the structure taking place gradually in the nucleus. The coiled aspect of the chromonemata becomes then more and more conspicuous, and the whole chromosomes can be traced for some considerable length.

3) The disturbance in the uniformity of the nuclear structure is due to the contraction or condensation of the chromosomes which are previously in the diffuse state. At the maximum condensation, the chromosomal bodies are distinguishable clearly from one another, the interchromosomal clear spaces being distinct, though a certain complication prevents us from the exact counting of their number. This stage correspond with the stage known as the "spiral stage" in somatic mitosis.

4) The condensed chromosomes are unravelled and the fine leptotene threads come out of them. The unravelling takes place without showing any marked change in the thickness of the chromonema threads in striking contrast to the case of the somatic mitosis where the threads become remarkably thicker than before unravelling.

5) The "Ansammlung der Chromatinkörner" seen in older literature seems to correspond with the chromosome condensation which we understand as the first stage observable distinctly in meiosis.

The writer wishes to acknowledge his sincere gratitude to Prof. Y. KUWADA for his kind guidance and encouragement throughout the course of the present investigation.

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Studies of Mitosis and Meiosis in Comparison IV. A contribution to the study of the origin of the "bouquet" and its formation[1]

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The formation of the bouquet is a characteristic feature in meiosis, but little is known as yet about its mechanism and origin. The thorough solution of the problem is hardly accessible to us yet, but this is a very important problem in the study of meiosis, because the bouquet is formed during the period in which syndesis is about to take place. In the course of the study of the early prophase of meiosis, certain facts were observed which may have a certain significance with respect to the origin and the formation of the bouquet. They are briefly reported below.

Material and Method

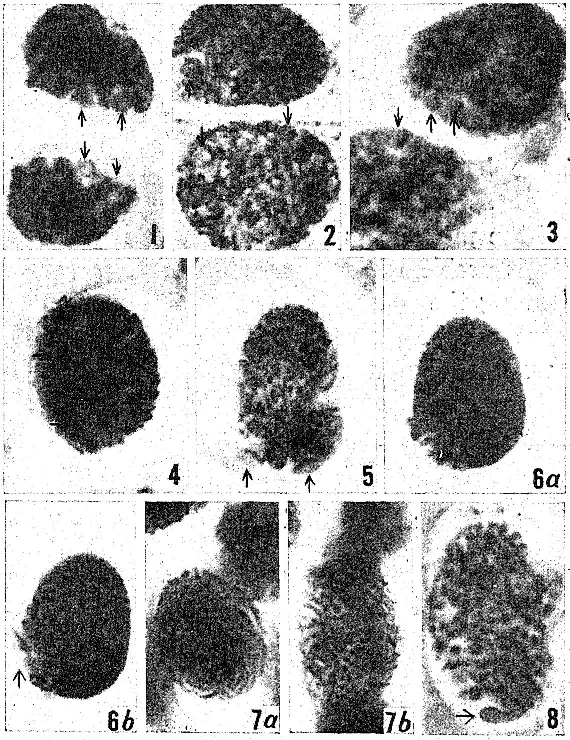
Tradescantia reflexa, *Trillium* sp. and *Psilotum* sp. were observed. In the cases of *Trillium* and *Psilotum*, the acetocarmine smear method was used, and in *Tradescantia* the observation was made with fixed material. Young flower buds were fixed with a modification diluted to 1/4 concentration of FLEMMING's stronger solution not containing any trace of acetic acid, after pretreatment with CARNOY's mixture for a few seconds, and the microtome sections were stained after FEULGEN's nucleal staining method, and double stained with light-green.

Observation

1. *Tradescantia reflexa.* In the interphase immediately preceding the meiosis, 5–6 nucleoli are found in the nucleus. When the nucleus enters into meiotic prophase and the spiral stage is reached (HIRAOKA, 1941), the number of the nucleoli becomes reduced, in most cases to 1–2. The nucleoli are now much larger in size than when found in the greater number, and are flattened in shape

1) Investigation supported by grant from the Science Research Fund of the Department of Education.

(Figs. 5, 6b). They are found attached to the nuclear membrane. At the "unravelling stage" or in the beginning of the leptotene stage, there becomes recognizable the tendency of parallel orientation of the chromosome threads. The threads gradually appear to run parallel, with their basal parts on the nuclear membrane to which the nucleoli are attached. In some cases, the parallel threads are found with their ends on the surface of the nucleolus (cf. KAUFMANN, 1925). This region where the nucleoli are found forms the basal part of the bouquet (Fig. 6a). The front view of the bouquet shows that at levels apart from its base the parallel threads present a whirling aspect like a vortex with the base in its center (Fig. 7a, b; cf. MIYAKE, 1905, Fig. 137; BĚLAŘ, 1928, Fig. 143). This is the leptotene bouquet. The chromosome pairing becomes visible first at the basal region where the nucleolus or nucleoli are found — the amphitene bouquet. The pairing proceeds gradually along the whole lengths of the chromosomes, the chromosome threads thus appearing much thicker—the pachytene bouquet (Fig. 8). At this stage the chromosomes show the spiral structure. Then the characteristic orientation of the chromosomes as a bouquet disappears, and the pachytene chromosome threads become evenly distributed in the nucleus without showing any special orientation. In



Figs. 1–8. *Tradescantia reflexa*. 1. Premeiotic telophase. 2, 3. Two succesive stages before spiral stage. 4. Spiral stage. 5. Unravelling stage or early leptotene. 6. Late leptotene or beginning of leptotene bouquet; *a*) showing the basal part of the bouquet and *b*) the same showing spindle shaped nucleolus at the base. 7. Leptotene bouquet; *a*) front view, *b*) side view. 8. Pachytene bouquet, side view. In all these figures except Figs. 6*a* and 7, the arrows indicate nucleoli.

the paraffin sections the contents of the nuclei at the spiral stage and later stages up to the pachytene bouquet are frequently found considerably contracted—the "first contraction" (Figs. 4–8)[1].

In the premeiotic divisions, it is observed that the nucleoli are formed in telophase at the distal region of the nucleus or the region on the side of the nucleus near the new cell wall, so that the nucleoli assume symmetrical positions in the two daughter nuclei in respect to the cell membrane in between (Fig. 1). This symmetrical distribution of the nucleoli is often recognizable also in the very beginning of the meiotic prophase (Figs. 2, 3), indicating that in the bouquet arrangement of chromosomes the basic region where the nucleoli are found is the distal chromosome region.

2. *Psilotum* sp. In this plant the bouquet is of the typical form without presenting any whirling aspect. The basal part of the bouquet is found on the nuclear membrane, but one or two nucleoli which were observed show no definite relation in position to the bouquet base. They may be found quite near the nuclear membrane, sometimes, at the base of the bouquet, taking symmetrical positions in the sister nuclei as in the case of *Tradescantia,* but may also assume any random positions. It is sometimes seen clearly that two chromosome threads probably representing the arms of a chromosome come out of a small chromatin mass found on the surface of the nucleolus (LATTER, 1926).

Although in this plant there is no definite relation between the position of the nucleoli and the base of the bouquet, we have some evidence to show that the region of the nucleus where the bouquet base is found is the distal one as in *Tradescantia.* In this plant the spore mother cells are found grouping together in early stages of meiotic prophase. In the spiral stage the number of the mother cells in a group is usually 8, and it is 4 in the later stages to about leptotene bouquet (Fig. 9) and 2 in the still later stages to the early pachytene (Fig. 10). The plane of contact of these two cells must correspond with the equatorial plane in the preceding division, and on this side of the nucleus the basal part of the bouquet is found, with some few exceptions which are probably due to the rotation of the nucleus from its original orientation (cf. JANSSENS, cited from WILSON, 1925). The region of the nucleus where the basal part of the bouquet is found must, therefore, be the distal one as in *Tradescantia.* At this stage, the nucleus is found rather displaced towards the distal region of the cell or the region on the

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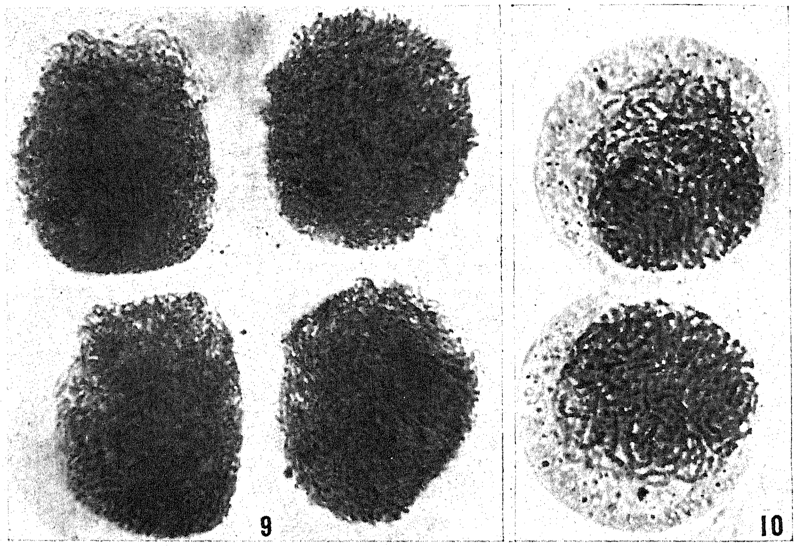
1) The method of preparation is responsible for the degree of nuclear contraction. In acetocarmine preparations, the nuclear substance is found less contracted than in the case of paraffin sections.

side next its sister cell, and accordingly starch grains are found more abundantly in the proximal or opposite side of the cell than in the distal region.

There is other evidence to show that the basal part of the bouquet corresponds with the distal region of the nucleus. This is not only an additional piece of evidence for the question at issue, but also very important in connection with the question of the mechanism of the bouquet formation. The evidence concerns the physical or chemical peculiarities of a part of the cell and nuclear membranes.



Figs. 9, 10. *Psilotum* sp. **9.** Leptotene bouquet. **10.** Pachytene bouquet.

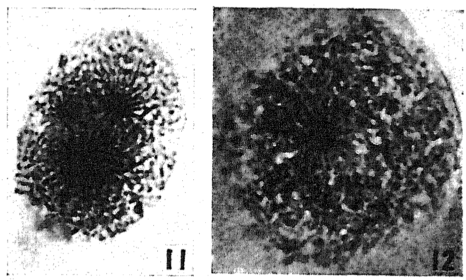
The part of the cell membrane along which the cell is in contact with its sister cell is dissolved by "cuoxam" more quickly than the remaining part of the membrane. To show an instance, in this part the membrane began to be dissolved 1–2 minutes after the reagent was applied, while in the remaining part it took abut 40 minutes before the dissolution began. This fact must show that this part of the membrane is younger in its history of formation than the other part, and accordingly that the conclusion drawn above that the region where two mother cells are in contact corresponds with the region of the equational plane in the preceding division should be correct.

In this part of the cell membrane in contact with the sister cell, plasmodesma are demonstrable, when it is treated with iodine sulphuric acid after fixing with iodine potassium iodide (TUNMANN,

1913). It is thus comprehensible that through this porous membrane the transposition of the nuclear material into the adjacent cell takes place as has been observed by many authors at this stage of meiosis.

The treatment with a solution of sodium carbonate shows also that there is a difference in the degree of swelling of the cell membrane between the part in contact with the adjacent cell and the other or the free part, the former swelling in a smaller degree than the latter, though no difference is recognizable as the result of staining with corallin soda and ruthenium red.

All these points mentioned above show that the physical and chemical properties of the cell membrane are different in different parts, according to whether or not the parts occupy the region corresponding with that lying between the two poles in the preceding division. Such a partial difference of the membrane in the cell wall in relation to the nuclear division is also recognizable in the case of the nuclear membrane, when a plasmolized cell is deplasmolized. When a cell at pachytene bouquet is treated first with a 20% solution of saccharose and then with a 50% solution of the same replacing the first solution, plasmolysis takes place. When the medium is then replaced with water, the nucleus swells and bursts, always at the region confronting the part of the cell membrane in contact with the adjacent cell, that is, therefore, at the part of the nuclear membrane at which the basal part of the bouquet is attached.



Figs. 11, 12. *Trillium* sp. 11. Leptotene asters. 12. Pachytene aster.

From the results of observation and experiments mentioned above, we are led to the speculation that the part of the nuclear membrane to which is attached the basal part of the bouquet, and which is characterized with a particular physical property by which this part is probably more easily permeable to water than the other part, plays a prominant rôle in taking water in or out of the nucleus which is activated to meiosis, and that this particular condition of the nuclear membrane at the base of the bouquet should take some part in the formation of the bouquet arrangement of the chromosome threads.

3. *Trillium* sp. In this plant, several (1–4) asteric figures which in appearance very much remind us of the cytasters observed in the artificial parthenogenesis in animals, are found in the nucleus

at the stages from leptotene to amphitene or early pachytene (Fig. 12, comp. Fig. 11). The asters consist of the chromosome threads, and at the center of the aster a clear space may sometimes be observed, though this is not frequently the case. It seems likely that in this case the bouquet is divided into several partial bouquets with pointed bases instead of one diffuse base as in *Psilotum*. Two partial bouquets often present aspects as of two asters fusing together. In some pollen mother cells, all the partial bouquets, and less frequently, some of them, present such a whirling aspect as that observed in *Tradescantia*, the chromosome threads appearing to run parallel in side view.

The behaviour of the nucleolus in relation to the formation of the asters has not yet been clearly studied. The relation of the chromocenters to the aster formation which relation may also exist between them is also left for a further investigation.

Conclusion

In the higher plants, the occurrence of the bouquet has been reported in rather few cases. KAUFMANN (1925) has observed that in *Tradescantia pilosa* the spiremes loop out in the nuclear cavity from the "attachment plate" found on one side of the nucleus. KIHARA (1927) has observed that in *Rumex acetosella* the fine chromatin threads oriented towards a pole gradually contract and form the leptotene bouquet, at the polarization pole of which the approximation of the homologous chromosomes begins to take place. BĚLAŘ (1928) gives photomicrographs of the amphitene bouquet in *Tradescantia virginica* in his Fig. 143. In this figure, he explains that the proximal ends of the chromosomes are brought together into a bundle which stands normally on the nuclear membrane, and that the distal ends wind themselves round the bundle. According to NEBEL and RUTTLE (1936), the bouquet or "whorl stage" precedes the leptotene in *Tradescantia*. ATWOOD (1937) describes the bouquet stage in *Gaillardia*, and considers the regular orientation of the threads "to be directly related to their regular orientation in the anaphases and telophases of the last premeiotic division."

In the present investigation it is demonstrated that the base of the bouquet corresponds with the distal parts of the chromosomes or, at least, is found at the distal region of the nucleus, by the distribution of the nucleoli in the case of *Tradescantia*, and by the arrangement of the sister spore mother cells and the partial difference in physical and chemical properties of the cell and nuclear membranes in the case of *Psilotum*. In *Tradescantia* it is observed

that the chromosome pairing begins at the basal part of the bouquet. A direct conclusion is, therefore, that in this plant the chromosomes associate in pairs first at their distal ends, not at their attachment constrictions.

In the case of a bouquet being formed, there seem to be two opinions as to the question at what part of the chromosomes the pairing begins. According to one opinion, it begins at the proximal part of the chromosomes and according to the other it does so at the distal part. According to WENRICH (1917), in *Chorthippus* the parallel conjugation of the prophase threads takes place beginning at the proximal part of the leptotene threads. In his Figs. 2, 3 and 4, Pl. III, the parallel arrangement of the chromosomes two by two is seen in the basal part of the bouquet, indicating that in this case the conjugation begins at this parts of the bouquet, and hence that the bouquet base must correspond with the proximal part of the chromosomes. If in *Tradescantia virginica* (BĚLAŘ, l.c.) the chromosome pairing begins at the bouquet base as inferable in the case of *Chorthippus*, and if in this plant the proximal parts of the chromosomes form the bouquet base as explained by BĚLAŘ, we must then conclude that in the case of *Tradescantia virginica* too the chromosome pairing begins at the proximal part of the chromosomes.

According to ROBERTSON (1931), in *Acridium* and in some other Orthoptera the process of the chromosome pairing begins at the distal ends of the chromosomes. His Figs. 36–38 appear to show that in this case too the pairing begins at the bouquet base, hence the bouquet base must correspond with the distal part of the chromosomes. According to JANSSENS (cited from WILSON, 1925), in a salamander the nucleus rotates through 180° after the final gonial division. If it rotates, the drawings reproduced by WILSON in his Fig. 278 F seem to show that the bouquet base corresponds with the distal end of the chromosomes, though in this figure the structure suggesting the bouquet base is somewhat obscure. GELEI (1922) has observed that the central body travels to the opposite side of the nucleus after the final gonial division giving rise to the same effect as the rotation of the nucleus through 180°. In his Figs. 28a and 29a, the central body is found on the side of the base of the bouquet. If in these cases the translocation of the central body has taken place, the bouquet base must represent the distal end of the chromosomes.

According to DARLINGTON (1935), in *Fritillaria* (root tip) "the chromosomes in prophase retain their arrangement with the spindle attachments pointing to the poles of the previous telophase". This description implies that the nucleus does not rotate before the prophase is reached. If in *Tradescantia* and *Psilotum* which we studied

the nucleus immediately preceding the meiosis does not rotate before the bouquet is formed as in the root tips in *Fritillaria*, the bouquet base will, in our cases, correspond with the distal ends of the chromosomes. If the nucleus rotates as observed in some animals, it will correspond with the proximal ends of the chromosomes. We have no positive evidence to judge which case is correct, but we may say that in either case the chromosome pairing begins at the basal part of the bouquet. It seems to be the fact of coincidence in all the cases mentioned above, that the chromosome pairing begins at the bouquet base whether it represents the proximal or the distal end of the chromosomes. DARLINGTON (1937) writes in his "Recent Advances": "With a bouquet arrangement pairing begins at the end or ends lying towards the surface of the nucleus, whether the centromere is situated there or not". If the chromosome pairing begins to take place at the bouquet base irrespective of whether it represents the proximal or distal chromosome ends, there should exist some significant relation between the bouquet formation and the initiation of the chromosome pairing. This conclusion seems likely when we consider the fact that in the case where the bouquet is absent, the chromosome pairing takes place more or less irregularly.

DARLINGTON (1937) states that "there can be no doubt of the absence of polarisation in many Lepidoptera, and in some Liliaceae except merely as a relic of the orientation of the chromosomes, with their centromeres towards the pole, at the preceding telophase". The fact that even in those cases where the polarization is not clearly observable, the relic of the telophasic orientation is recognizable is a very important fact in considering the origin of the bouquet. That in the bouquet its base corresponds with the proximal or distal ends of chromosomes must also show that the polarized orientation has its origin in the preceding telophase orientation as in some Liliaceae. In the formation of the bouquet, there must be other causes or conditions which bring this orientation into clearer view. In the case of *Psilotum* it seems highly probable that the differential structure of the nuclear membrane is one of these conditions. It seems probable that in association with the change in water relation in the nucleus in the prophase, this structural difference in the parts of the nuclear membrane is concerned in bringing the diffuse appearance presented by the nucleus before the bouquet stage into a clear polarized appearance. The various conditions at this stage may be different in different organisms, and accordingly the whirling aspect or somewhat convoluted aspect may be produced. If in the latter case a mass-contraction takes place, this will be the typical case of synizesis.

The fusion of the nucleoli observed in the case of *Tradescantia* may play some rôle in bringing the chromsomes together at the basal part of the bouquet. The behaviour of chromocenters may play a similar rôle, but may also play a rôle in the formation of several bundles of chromosomes or partial bouquets as observed in *Trillium*, though we have as yet no positive evidence to show this.

Summary

1) In *Tradescantia* and *Psilotum*, results are obtained which indicate that the base of the bouquet corresponds with the distal region of the chromosomes, if there takes place no rotation of the nucleus before the bouquet formation.

2) In *Psilotum*, it is shown by the deplasmolysis experiments, that the part of the nuclear membrane at the base of the bouquet is physically weaker than the other parts of the membrane.

3) In *Trillium*, it is frequently observed that the bouquet figure is like a group of cytasters.

4) It is suggested that the bouquet arrangement of the chromosome threads originates in the chromosome arrangement in the preceding telophase, and that in the presentation of the pronounced polarized appearance of the bouquet certain conditions or phenomena such as the differential structure of the nuclear membrane or the fusion of the nucleoli are concerned, in association with the change in water relation in the nucleus in prophase.

The present investigation was carried out under the direction of Prof. KUWADA to whom the author wishes to take this opportunity of expressing his sincere thanks. The author's thanks are also due to Dr. SINKE who helped the author much in various ways.

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Genomanalyse bei *Triticum* und *Aegilops*

von
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VIII. Rückkreuzung des Bastards *Ae. caudata* × *Ae. cylindrica* zu den Eltern und seine Nachkommen[1]

von
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Einleitung

In der VII. Mitteilung dieser Serie wurde über den Bastard *Ae. caudata* L. ($n=7$) × *Ae. cylindrica* HOST ($n=14$) und seine Rückkreuzung zu *Ae. caudata* kurz berichtet (KIHARA, 1937ab, 1940). In der I. Reifungsteilung des F_1-Bastards treten nämlich 7 Paare und 7 Univalente auf. Die Bivalenten sind sehr fest gebunden, wie bei der reinen Art *Ae. caudata*. 1–2 Trivalente sind oft zu beobachten. Die Univalenten werden zum Teil in der I. Anaphase längsgeteilt. Die so entstandenen Spalthälften werden in der II. Reifungsteilung ohne weitere Längsteilung auf die beiden Pole verteilt. Das Verhalten der Univalenten folgt somit dem Modus des *Pilosella*-Typs. Restitutionskerne und unreduzierte Gameten werden in diesem Bastard *Ae. caudata* × *Ae. cylindrica* nicht beobachtet.

Tabelle 1. Kreuzungserfolg bei den Rückkreuzungen des Bastards *Ae. caudata* × *cylindrica* zu den Eltern

Kreuzung	Zahl d. bestäubten Blütchen	Zahl d. Körner	Gekeimt	Kreuzungserfolg (%)
F_1♀ × *Ae. caudata*♂	220	8	2	0.90
F_1♀ × *Ae. cylindrica*♂	232	5	1	0.43

Der Bastard hat 0.5%ige gute Pollenkörner und zeigt nur 0.07%ige Fruchtbarkeit bei freiem Blühen. Die Rückkreuzung zu den beiden Eltern weist einen ziemlich guten Körneransatz auf (Tab. 1). Bei der Verbindung mit *Ae. caudata* als Pollen wurden 2 Pflanzen mit 7_{II} erhalten (Kultur–Nr. 230 und 231 im Jahre 1936).

1) Contributions from the Laboratory of Genetics, Biological Institute, Department of Agriculture, Kyoto Imperial University, No. 120.

Eine Pflanze (Nr. 235) auf Grund der Verbindung $F_1 \times$ *Ae. cylindrica* hatte 27 somatische Chromosomen. Die Homologie des Genoms zwischen *Ae. caudata* und *Ae. cylindrica* sowie das Verhalten der Chromosomen in der Nachkommenschaft der Rückkreuzung sollen im folgenden beschrieben werden.

Nachkommen der Rückkreuzung

1. $F_1 \times$ *Ae. caudata* und Nachkommen

Chromosomen und Fruchtbarkeit. Unter 2 Individuen von $F_1 \times$ *Ae. caudata* wies eine Pflanze (Nr. 231) bei freiem Blühen hohe Fruchtbarkeit (44.3%)[1] auf, ähnlich wie bei der reinen Art *Ae. caudata* (46.5%), die der anderen (Nr. 230) betrug nur 4.5%. Bei Isolierung zeigte aber Nr. 231 bzw. Nr. 230 eine 15.9-bzw. 6.3%ige Fruchtbarkeit. Auch der Prozentsatz der guten Pollen war bei der ersteren Pflanze 98%, bei der letzteren 80%.

Weil diese zwei Pflanzen 7 normale Paare aufwiesen, müßten eigentlich die 7-chromosomigen Gameten des F_1-Bastards normal funktionsfähig sein. Wenn man annimmt, daß die Verteilungsweise der 7 Univalenten bei der Reifungsteilung dem Zufall folgt und wenn man von einer Elimination absieht, so kann theoretisch nur 1 von 128 ($1/2^7$) Gameten 7-chromosomig sein. Der Prozentsatz der 7-chromosomigen Gameten beträgt demnach 0.78%. Wenn man aber eine Elimination der Univalenten in Rechnung zieht, wie z. B. beim pentaploiden *Triticum*-Bastard (KIHARA und MATSUMURA, 1940), so möchte dieser Prozentsatz etwas steigen. Daher stimmt das Verhältnis der funktionsfähigen 7-chromosomigen Gameten (0.90%, Tab. 1) mit dem erwarteten gut überein.

Die 7-chromosomigen Gameten von *Ae. caudata* $\times$ *Ae. cylindrica* entstehen durch die Rekombination der gepaarten Chromosomen der beiden Eltern. Unter diesen Gameten hat theoretisch 1 von 128 alle 7 Chromosomen von *Ae. caudata*[2]. Dieser Fall tritt demnach nur bei 0.006% ($1/2^7 \times 1/2^7 = 1/2^{14}$) von sämtlichen Gameten ein. Falls man annimmt, daß nur solche Gameten fertil sind, findet man einen merklich großen Unterschied zwischen dieser berechneten und der gefundenen Fruchtbarkeit (0.90%).

Unter der Nachkommenschaft von Nr. 230 und 231 hatten alle diejenigen Pflanzen, bei denen man die Reifungsteilung beobachten konnte, 7 Bivalente. In Tabelle 2 findet sich die Fruchtbarkeit dieser Nachkommen wiedergegeben, wobei man von den sterilen bis hoch

1) Die Bestimmung der Fruchtbarkeit fand bei freiem Blühen am 1. und 2. Blütchen statt, wenn nichts anderes bemerkt ist.

2) Von dem „Crossing-over" haben wir hier abgesehen.

fertilen Pflanzen ein starkes Variieren beobachten kann. Die Mode der Variationskurve liegt aber bei 50–55%. Im großen und ganzen läßt sich sagen, daß die meisten Pflanzen mittelmäßig fruchtbar sind, wie bei der reinen Art *Ae. caudata*.

Tabelle 2. Fruchtbarkeit der 14-chromosomigen Nachkommen von Nr. 230 und 231 ($F_1 \times$ *Ae. caudata*) im Jahre 1937

Kultur-Nr.	Fruchtbarkeit (%)																	
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85
230	2	1		2	1	2		2	3	4	4	4	1	2				
231	11	5	6	7	2	4	10	11	18	11	18	16	16	15	6	2	1	
Summe	13	6	6	9	3	6	10	13	21	15	22	20	17	17	6	2	1	

Bei der Kreuzung von Nr. 231 × *Ae. caudata* wurden im Jahre 1937 5 Pflanzen mit durchschnittlich 48.7%iger Fruchtbarkeit erhalten, von denen bei 4 die Chromosomenzahlen der Reifungsteilung beobachtet wurden und alle 7 Bivalente hatten. Die Fruchtbarkeit des gleichen Jahres der reinen Art *Ae. caudata* zeigte 30.3% (isoliert).

Bei *Aegilops*, besonders *Ae. caudata*, üben im allgemeinen die äußeren Bedingungen einen großen Einfluß auf die Fruchtbarkeit aus. Die meisten Ähren mit weniger als 20%iger Fruchtbarkeit in Tabelle 2 waren zu jung oder vermodert. Zum Vergleich sei die Fruchtbarkeit der reinen Arten *Ae. caudata* und *Ae. cylindrica* in Tabelle 3 herangezogen. Weil der Juni und Juli des Jahres 1938 regnerisch waren, zeigte sich in diesem Jahre die niedrigste Fruchtbarkeit, während man im Jahre 1940 die höchste Fertilität erhielt.

Tabelle 3. Fruchtbarkeit von *Ae. caudata* und *Ae. cylindrica* in den Jahren 1936–1940

Jahr	Fruchtbarkeit (%)	
	Ae. caudata	*Ae. cylindrica*
1936	46.5	54.3
1937	(30.3)[1]	(56.6)[1]
1938	12.7 (4.2)[1]	(45.8)[1]
1939	(29.8)[1]	93.3 (57.6)[1]
1940	(32.9)[1]	(64.6)[1]

1) Isoliert

Auf Grund dieser Ergebnisse ist man wohl berechtigt zu schließen, daß Nr. 230 mit niedriger Fruchtbarkeit eine durch äußere Faktoren bedingte Variante darstellt. Bei der Pollenanalyse von Nr. 230 und 231 wurden, wie oben erwähnt, 80% oder mehr gute Pollen beobachtet. Tabelle 2 zeigt auch, daß die Nachkommen beider Pflanzen die gleiche Fruchtbarkeit aufweisen.

Vererbung der morphologischen Eigenschaften. Bei der Nachkommenschaft von Nr. 230 trat eine Aufspaltung der Keimlinge im Verhältnis von 3 normal zu 1 *xantha* zutage. Auch bei Nr. 231 wurde das einfaktorielle Zahlenverhältnis zwischen normal und

albino festgestellt. Diese Spaltungsverhältnisse bestätigen sich durch die der Folgegenerationen. Zwei Eigenschaften, d.h. die Begrannung der Seitenährchen und Dauer bis zur Reife, zeigten je einfaktorielle Vererbung. *Ae. caudata* ist in allen Seitenährchen lang begrannt (Abb. 1, *a*) und reift spät, während *Ae. cylindrica* (mit Ausnahme des obersten Ährchens) unbegrannt und früh reifend ist. Beide Eigenschaften gewannen beim F_1-Bastard intermediären Ausdruck. Nr. 230 und 231 waren begrannt, ebenso wie bei *Ae. caudata,* und die Dauer bis zur Reife war bei beiden Pflanzen intermediär wie bei F_1. Bei einem Viertel der Nachkommenschaft beider Pflanzen spaltete sich die unbegrannte Pflanze auf (Abb. 1, *b*). Die Dauer bis zur Reife betreffend, war es oft etwas schwer, die intermediäre Dauer von der späten zu unterscheiden. Bei den Nachkommen stellten sich die erwarteten Kombinationen in folgendem Verhältnis dar: 111 begrannt spät (inkl. intermediär): 36 unbegrannt spät (inkl. intermediär): 27 begrannt früh: 5 unbegrannt früh. Hieraus geht klar hervor, daß diese beiden Eigenschaften unabhängig voneinander vererbt werden.

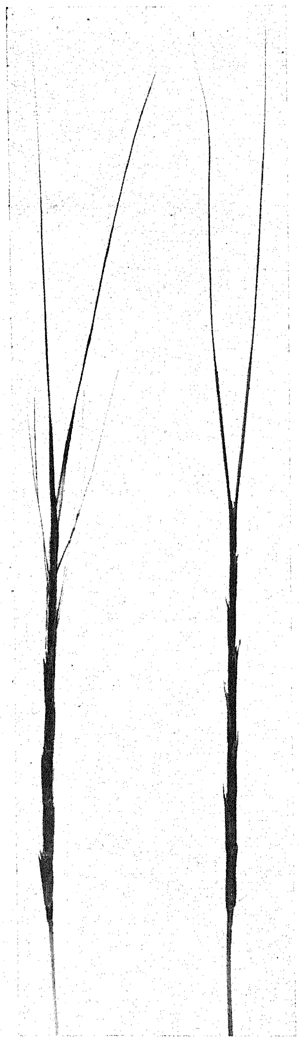

Abb. 1. Ähren von *Ae. caudata* (*a*) und einem unbegrannten Nachkommen von $F_1 \times$ *Ae. caudata* (*b*). Vergr. 3/5.

Homologie des Caudata-Genoms mit einem Genom von Ae. cylindrica. Den oben dargelegten Untersuchungen nach können die folgenden Befunde zum Beweise der Homologie des *Caudata*-Genoms mit einem der zwei Genome von *Ae. cylindrica* dienen.

1) Der Bastard *Ae. caudata* $\times$ *Ae. cylindrica* hat $7_{II} + 7_{I}$ Chromosomen bei der Reifungsteilung.

2) Die gefundene Häufigkeit der funktionsfähigen 7-chromosomigen Gameten des F_1-Bastards steht im großen und ganzen im Einklang mit der theoretischen.

3) Bei der Rückkreuzung $F_1 \times$ *Ae. caudata* und ihrer Nachkommenschaft ergab sich eine normal fertile Pflanze mit 7 Bivalenten, die in jeder Hinsicht mit *Ae. caudata* vollkommen identisch war.

4) Ferner ist betreffs der morphologischen Eigenschaften dieser Pflanzen mit 7 Bivalenten die einfache MENDELsche Vererbung anzunehmen.

Das Resultat der Karyotypanalyse stimmt vollkommen mit demjenigen der Genomanalyse überein (SENJANINOVA-KORCZAGINA, 1932).

Die genomatische Formel für *Ae. cylindrica* wurde bereits in der II. Mitteilung durch CCDD ausgedrückt (KIHARA, 1931). Weil *Ae. caudata* kein mit der Dinkelreihe des Weizens (ABD) homologes Genom besitzt (vgl. VI. Mitt.), muß das gemeinsame Genom von *Ae. caudata* und *Ae. cylindrica* C sein. Das C-Genom von *Ae. caudata* sei mit dem Buchstaben C_{caud} und das von *Ae. cylindrica* mit C_{cyl} bezeichnet (KIHARA, 1940). Ersteres ist völlig austauschbar mit C_{cyl}, d.h. die Rekombination zwischen beiden Genomen hat keinen Einfluß auf die Fruchtbarkeit. Die bifaktorielle Spaltung der morphologischen Eigenschaften muß wirklich auf einer Rekombination von zwei bestimmten Chromosomen beruhen. Bei der für die Untersuchung gebrauchten Art (*Ae. caudata* L. var. *polyathera* BOISS.) sind die Seitenährchen lang begrannt, während eine andere Varietät (var. *dichasians* ZHUK.), welche in unserem Sortimente lange fehlte, unbegrannt ist. Aus der Rückkreuzung $F_1 \times$ *Ae. caudata* var. *polyathera* ist es uns tatsächlich gelungen, die Varietät *dichasians* neu zu erzeugen. Die neu hervorgebrachte unbegrannte Rasse unterscheidet sich dadurch, daß sie sich etwas zugespitzt bis kurz begrannt zeigt, von der alten völlig unbegrannten Rasse.

2. F_1 × *Ae. cylindrica* und Nachkommen

Schwankung der Chromosomenzahl und Fruchtbarkeit. Bei der Rückkeuzung mit dem 28-chromosomigen Elter *Ae. cylindrica* wurde eine Pflanze (Nr. 235) im Jahre 1936 erhalten, die 27 somatische

Tabelle 4. Häufigkeit der verschiedenen Kombinationsweisen der meiotischen Chromosomen in den PMZ aus 27-chromosomigen Pflanzen (Nr. 235 und ihre Nachkommen)

Nr. der Pflanze	Kombinationsweise						Summe
	$1_{III}+12_{II}$	$13_{II}+1_I$	$12_{II}+3_I$	$11_{II}+5_I$	$1_{III}+11_{II}+2_I$	$2_{III}+10_{II}+1_I$	
Nr. 235	22	11	7	—	—	—	40
Nachkommen	19	22	11	1	2	2	57
Summe	41	33	18	1	2	2	97

Chromosomen hatte (Tab. 1). In der I. Metaphase trat bei Nr. 235 meist $1_{III}+12_{II}$ auf. Außer dieser Konfiguration wurden $13_{II}+1_I$ und $12_{II}+3_I$ beobachtet. Tabelle 4 zeigt die Häufigkeit der Kon-

jugationsmodi bei den 27-chromosomigen Individuen (Nr. 235 und ihr gleichchromosomiger Nachkomme). Vereinzelt wurden die Konfigurationen $2_{III}+10_{II}+1_{I}$, $1_{III}+11_{II}+2_{I}$, $11_{II}+5_{I}$ bei den Nachkommen festgestellt.

Bei der Rückkreuzung Nr. 235 ♀ × *Ae. cylindrica* ♂ wurden ferner 8 27- und 2 28-chromosomige Individuen konstatiert (Tab. 5). Die Nachkommen von Nr. 235 waren meistens 27- oder 28-chromosomig; nur eine 29-chromosomige Pflanze war darunter (Tab. 6).

Tabelle 5. Häufigkeit der Pflanzen mit verschiedenen Chromosomenkombinationen in der Kreuzung Nr. 235 × *Ae. cylindrica* und ihre Fertilität

Kombinationsweise	$1_{III}+12_{II}$	$13_{II}+1_{I}$	14_{II}	$1_{IV}+12_{II}$	Summe
Anzahl der Pflanzen	6	2	1	1	10
Fertilität (%)	48.8	41.4	(62.5)[1]	65.1	

1) Isoliert.

Tabelle 6. Häufigkeit der Pflanzen mit verschiedenen Chromosomenkombinationen in der Nachkommenschaft von Nr. 235 und ihre Fertilität

Kombinationsweise	$1_{III}+12_{II}$	$13_{II}+1_{I}$	14_{II}	$1_{IV}+12_{II}$	$13_{II}+1_{I}$ +fr.	$14_{II}+1_{I}$	Summe
Anzahl der Pflanzen	11	7	11	5 + 1[2]	1	1	37
Fertilität (%)	41.2	36.2	60.0	49.5 8.3	32.6	(80.8)[1]	

1) Isoliert.
2) Ein Chromosom des tetrapartiten Komplexes ist klein; manchmal werden ein Tripartites und ein Fragmentchromosom beobachtet.

Unter den 27-chromosomigen Nachkommen konnten wir der Chromosomenkombination nach 2 Kategorien unterscheiden. Die erste hat meist ein Tripartites, das oft $1_{II}+1_{I}$ oder 3_{I} bildet, wie bei Nr. 235 (vgl. Tab. 4). Diese Pflanze sei einfachheitshalber mit $1_{III}+12_{II}$ bezeichnet. Das Tripartite war meist V-förmig, doch trat hier, wenngleich selten, auch die Y-förmige Anordnung auf. Bei der zweiten wurde kein Tripartites gefunden; sie wies die Konfiguration $13_{II}+1_{I}$ (selten $12_{II}+3_{I}$) auf.

Tabelle 7. Häufigkeit der verschiedenen Kombinationsweisen der meiotischen Chromosomen in den PMZ aus einer Pflanze mit $1_{IV}+12_{II}$

$1_{IV}+12_{II}$	14_{II}	$1_{III}+12_{II}+1_{I}$	$13_{II}+2_{I}$	Summe
20	19	8	3	50

Die 28-chromosomigen Nachkommen lassen sich wieder in zwei Gruppen einteilen. Die eine ist dadurch charakterisiert, daß sie oft ein Tetrapartites hat, die andere dadurch, daß das Komplexchromosom fehlt. Bei der ersteren Gruppe wurde meist die Konfiguration $1_{IV}+12_{II}$ oder 14^{II} beobachtet, selten dagegen $1_{III}+12_{II}+1_{I}$ und nur vereinzelt $13_{II}+2_{I}$ (Tab. 7). Wir wollen

diese Pflanzen einfachheitshalber mit $1_{IV} + 12_{II}$ bezeichnen. Im allgemeinen wurde das N-förmige Tetrapartite beobachtet; oft trat auch der U-förmige Komplex auf. Bei den Pflanzen mit nur 14_{II} wurde selten eine geringe Anzahl abweichender Chromosomenkombinationen $13_{II} + 2_{I}$ gefunden, wie bei der reinen Art *Ae. cylindrica.* Ausnahmsweise hatte eine 28-chromosomige Pflanze fast immer $13_{II}+2_{I}$, wo das eine univalente Chromosom wahrscheinlich ein Chromosomenfragment darstellte und deutlich kleiner war als das andere. Ein Chromosom des tetrapartiten Komplexes war bei einer Pflanze mit $1_{IV}+12_{II}$ auch so klein, so daß wir diesen Komplex vom tripartiten nicht leicht zu unterscheiden vermochten. Doch löst sich dieser Komplex oft in ein Tripartites und ein kleines Univalentes auf. Beim 29-chromosomigen Nachkommen wurden die Konfigurationen $14_{II} + 1_{I}$ und $1_{III} + 13_{II}$ beobachtet.

Nr. 235 hatte nur 10.3%ige Fruchtbarkeit. Die Fertilität bei der Rückkreuzung bzw. bei der Nachkommenschaft aus Selbstbestäubung im Jahre 1937 ist aus Tabelle 5 bzw. 6 zu ersehen. Im allgemeinen wiesen die Pflanzen mit verschiedener Chromosomenkombination in der Rückkreuzung eine höhere Fruchtbarkeit auf als in der Nachkommenschaft aus Selbstbestäubung. Bei der Rückkreuzung besitzen alle Pflanzen mindestens zwei vollständige Genome von *Ae. cylindrica* (CD), was ihnen eine höhere Fertilität sichert.

Eine Pflanze mit $14_{II} + 1_{I}$ zeigte höchste Fruchtbarkeit. Die Fruchtbarkeit der Pflanzen mit 14_{II} war auch hoch, ebenso wie bei der reinen Art *Ae. cylindrica* (Tab. 3). Die Pflanzen mit $1_{IV} + 12_{II}$ wiesen hingegen eine niedrigere Fruchtbarkeit auf als die mit 14_{II}. Ferner war eine Pflanze mit $13_{II} +1_{I} +1$fr. niedrig fertil (32.6%) und eine mit $12_{II} +1_{IV}$ (einem kleinen Chromosom des Komplexes) zeigte eine ganz geringe Fertilität (8.3%). Die 27-chromosomigen Pflanzen waren im großen und ganzen weniger fertil als die 28-chromosomigen. Unter 27-chromosomigen Individuen zeigte $1_{III} +12_{II}$ auch eine etwas höhere Fruchtbarkeit als $13_{II} +1_{I}$.

Nachkommen der 27-chromosomigen Pflanzen. Tabelle 8 bringt die Chromosomenzahlschwankung in der weiteren Generation der 27-chromosomigen Pflanzen im Jahre 1938. Aus einer Pflanze mit

Tabelle 8. Häufigkeit der Pflanzen mit verschiedenen Chromosomenkombinationen in der Nachkommenschaft der 27-chromosomigen Pflanzen im Jahre 1938

Eltern	Kombinationsweise				Summe
	$1_{III}+12_{II}$	$13_{II}+1_{I}$	14_{II}	$1_{IV}+12_{II}$	
$1_{III}+12_{II}$	5	0	6	4	15
$13_{II}+1_{I}$			8		8

$1_{III}+12_{II}$ ergaben sich Individuen mit $1_{III}+12_{II}$, $13_{II}+1_{I}$, 14_{II} und $1_{IV}+12_{II}$, ähnlich wie bei Nr. 235 (vgl. Tab. 6), während bei der Pflanze mit $13_{II}+1_{I}$ alle Individuen zu dem tetraploiden Zustande zurückkehrten.

Nachkommen der 28-chromosomigen Pflanzen. Die Pflanzen mit 14_{II} zeigten immer die konstante Chromosomenzahl 28. Bei der Nachkommenschaft der Pflanzen mit $1_{IV}+12_{II}$ ergaben sich in den Jahren 1938–1940 33 Pflanzen mit 14_{II} und 55 mit $1_{IV}+12_{II}$ (Tab. 9). Das

Tabelle 9. Häufigkeit der Pflanzen mit verschiedenen Chromosomenkombinationen in der Nachkommenschaft der 28-chromosomigen Pflanzen in den Jahren 1938–1940 und ihre Fertilität

Jahr	Eltern	Kombinationsweise				Summe
		14_{II} (Fertilität %)	$1_{IV}+12_{II}$ (Fertilität %)	$14_{II}+1_{I}$	$13_{II}+1_{I}$	
1938	14_{II}	3				3
	$13_{II}+1_{I}+$fr.	1				1
	$1_{IV}+12_{II}$	16	23	1		40
	14_{II} ($1_{IV}+12_{II}$)[1]				3	3
1939	14_{II}	3 (74.6)				3
	$1_{IV}+12_{II}$	9 (81.3)	16 (67.1)			25
	14_{II} ($1_{IV}+12_{II}$)[1]	11[2] (78.2)				11
1940	$1_{IV}+12_{II}$	8 (77.8)	16 (71.1)			24

1) Sehr selten wurde die Konfiguration $1_{IV}+12_{II}$ beobachtet und nur manchmal $13_{II}+2_{I}$.
2) Bei einigen Pflanzen trat manchmal die Konfiguration $13_{II}+2_{I}$ auf.

Verhältnis ist demnach ungefähr 1:2>. Bei einigen Pflanzen mit $1_{IV}+12_{II}$ trat in den Jahren 1939–1940 das O-förmige Tetrapartite außer dem N- oder U-förmigen auf. Abbildung 2, *b–d* zeigt die verschiedenen Chromosomenkombinationen einer der oben beschriebenen Pflanzen. Bei der Nachkommenschaft einer Pflanze mit $12_{II}+$④[1] wurden ferner bis 2 Komplexchromosomen beobachtet; die Konfigurationen waren z.B. $2_{IV}+10_{II}$, $1_{IV}+1_{III}+10_{II}+1_{I}$ usw.. 2 Tetrapartite zeigten einmal „interlocking" (Abb. 2, *f*). Aus den Rückkreuzungen von $1_{IV}+12_{II}\times$ *Ae. cylindrica* ergaben sich im Jahre 1940 14 Pflanzen mit 14_{II} (Abb. 2, *a*) und 17 mit $1_{IV}+12_{II}$ (Abb. 2, *e*) (Tab. 10). Ihr Verhältnis ist demnach ungefähr 1:1. Im allgemeinen waren die Pflanzen mit 14_{II} etwas fertiler als die mit $1_{IV}+12_{II}$ (Tab. 9 und 10).

Tabelle 10. Häufigkeit der flanzen mit verschiedenen Chromosomenkombinationen in der Kreuzung $1_{IV}+12_{II}\times$ *Ae. cylindrica* im Jahre 1940 und ihre Fertilität

Kombinationsweise	14_{II} (Fertilität %)	$1_{IV}+12_{II}$ (Fertilität %)
Anzahl der Pflanzen	14 (72.6)	17 (72.3)

1) ④ bedeutet ein O-förmiges Tetrapartites.

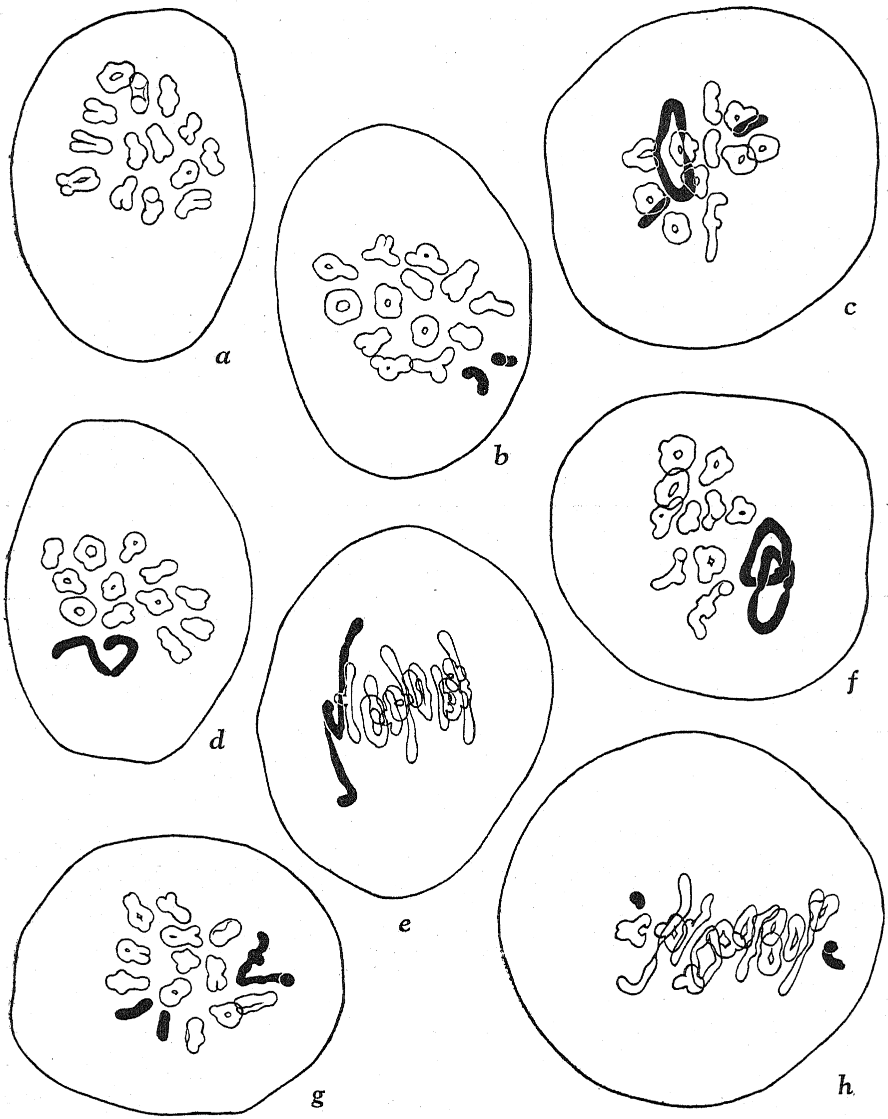

Abb. 2. I. Metaphase in PMZ der Pflanzen in der Nachkommenschaft von F_1 × *Ae. cylindrica*. Vergr. 1375.
Die Tri- und Tetrapartiten und Univalenten sind in sämtlichen Abbildungen schwarz.
a 14_{II}, *b* $13_{II}+2_I$, *c* ④$+11_{II}+2_I$, *d e* $1_{IV}+12_{II}$, *f* $2_{IV}+10_{II}$, *g* $1_{III}+12_{II}+2_I$, *h* $14_{II}+2_I$.

Eine Pflanze mit $13_{II}+1_I+1$fr. kehrte in der nächsten Generation zu dem tetraploiden Zustande 14_{II} zurück (Tab. 9). Bei der 28-chromosomigen wurden ausnahmsweise 2 Pflanzen gefunden, bei denen sehr selten die Konfiguration $1_{IV}+12_{II}$ außer 14_{II} beobachtet wurde, und zwar schied sich ein Bivalentes leicht in 2 Univalente.

Eine Pflanze mit solcher Kombination hatte ein U-förmiges Tetrapartites und stammte von einer Pflanze mit $1_{IV}+12_{II}$ (1937); ihre Nachkommen wiesen im Jahre 1938 alle $13_{II}+1_{I}$ auf. Eine andere Pflanze stammte von einer solchen mit $14_{II}+1_{I}$ (1938), in deren Nachkommenschaft 11 Pflanzen mit 14_{II} auftraten, von denen einige manchmal die Konfiguration $13_{II}+2_{I}$ hatten (Tab. 9).

Nachkommenschaft der 29- und 30-chromosomigen Pflanzen. Bei der Nachkommenschaft der Pflanze mit $14_{II}+1_{I}$ ($1_{III}+13_{II}$) wiesen die Pflanzen mit 14_{II} bedeutend viel mehr Vertreter auf, als die mit $14_{II}+1_{I}$ [manchmal $1_{III}+13_{II}$, selten $1_{III}+12_{II}+2_{I}$ (Abb. 2, *g*)] (Tab. 11). Die Fruchtbarkeit war aber im ersteren Falle im

Tabelle 11. Häufigkeit der Pflanzen mit verschiedenen Chromosomenkombinationen in der Nachkommenschaft der 29- und 30-chromosomigen Pflanzen in den Jahren 1938–1940 und ihre Fertilität

Jahr	Eltern	Kombinationsweise					Summe
		14_{II} (Fertilität%)	$1_{IV}+12_{II}$ (Fertilität%)	$14_{II}+1_{I}$ (Fertilität%)	15_{II} (Fertilität%)	$14_{II}+2_{I}$ (Fertilität%)	
1938	$14_{II}+1_{I}$	15 (31.5)	6 (31.2)	11 (36.7)	2 (4.9)	1 (15.3)	35
1939	$14_{II}+1_{I}$	3	1	1			5
	15_{II}			8			8
	$14_{II}+2_{I}$			1		1	2
1940	$14_{II}+1_{I}$	22 (80.4)	2 (86.6)	2 (83.9)			26

großen und ganzen niedriger als im letzteren. Ferner traten oft Pflanzen mit $1_{IV}+12_{II}$, selten solche mit 15_{II} oder $14_{II}+2_{I}$ auf. Die Pflanze mit 15_{II} wies die niedrigste Fruchtbarkeit (4.9%) auf; die Chromosomenzahl aller ihrer Nachkommen war unerwartet 29 und zeigte die Konfiguration $14_{II}+1_{I}$ ($1_{III}+13_{II}$). Aus einer Pflanze mit $14_{II}+2_{I}$, deren Fruchtbarkeit nur 15.3% war, erhielt man die Pflanzen mit $14_{II}+1_{I}$ und $14_{II}+2_{I}$ (Abb. 2, *h*).

Betrachtung über die Chromosomenzahlschwankung. Die 27-chromosomige Pflanze (Nr. 235) aus der Rückkreuzung $F_{I}\times$ *Ae. cylindrica* ist höchstwahrscheinlich durch die Verschmelzung einer 13-chromosomigen Eizelle des F_1-Bastards mit einem 14-chromosomigen Spermakern von *Ae. cylindrica* entstanden. Die Konjugationsweise der 27 Chromosomen weicht von der der normalen ($2n$-1)-Pflanzen ab, wo man immer $(n\text{-}1)_{II}+1_{I}$ beobachtet hat. Es besteht darüber kein Zweifel, daß es sich hier um ein Chromosom von den 13 Chromosomen aus F_1 handelt, welches mit 2 *Cylindrica*-Chromosomen 1_{III}, $1_{II}+1_{I}$ oder 3_{I} bildet.

Die 14 Chromosomen von *Ae. cylindrica* werde ich einfach mit 12 + ab + cd bezeichnen. Falls man annimmt, daß ein Chromosom

bc, welches aus einem Segmentaustausch zwischen ab und cd entstanden,[1] in der 13-chromosomigen Eizelle enthalten ist, lassen sich die Konfigurationen $12_{II} + 1_{III} \left(\begin{smallmatrix} ab & & cd \\ & \diagdown\diagup & \\ & bc & \end{smallmatrix}\right)$, $12_{II} + 1_{II} \left(\begin{smallmatrix} ab \\ | \\ bc \end{smallmatrix}\right) + 1_{I}(cd)$ oder $12_{II} + 3_{I}(ab + bc + cd)$ leicht erklären. Weiter sei angenommen, daß die Konjugation zwischen dem Chromosom ab und bc stärker als die zwischen bc und cd sei.

Indem wir diese 2 Voraussetzungen machen, wollen wir versuchen, die Schwankung und die Konjugationsweise der Chromosomen bei den Nachkommen von Nr. 235 zu demonstrieren. Die Gameten der 27-chromosomigen Pflanze haben demnach außer 12 normalen Chromosomen die folgenden, je nachdem sich die 3 Chromosomen ab, bc und cd verteilen:

1) $\dfrac{ab \quad cd}{bc}$ zahlreich, 2) $\dfrac{bc \quad cd}{ab}$ oft,

3) $\dfrac{ab \quad bc}{cd}$ selten, 4) $\dfrac{ab \quad bc \quad cd}{(\text{steril})}$ sehr selten.

Die erste Verteilung dürfte am häufigsten sein, während die vierte, wo die Gameten mit nur 12 Chromosomen wohl kaum funktionsfähig sind, sehr selten auftritt. Bei Anerkennung der 2. Voraussetzung ergibt es sich zweifellos, daß der zweite Fall etwas häufiger beobachtet wird als der dritte. Bei der Selbstbestäubung der 27-chromosomigen Pflanzen dürften die Gameten mit 12 + ab + cd bei der Konkurrenz in der Befruchtung überhand nehmen, besonders bei den Pollenkörnern.

Alle von Nr. 235 überhaupt mögliche Nachkommenschaft ist im Schema I zusammengestellt. Dieses wurde in so viele Quadrate eingeteilt, wie es der Häufigkeit der Nachkommen entspricht. Von den Kombinationen dürften die mit einem Sternchen nicht lebensfähig sein, da bei ihnen nämlich ein Chromosomsegment (a oder d) gänzlich fehlt. Tatsächlich dürften auch die mit kleinen Buchstaben bezeichneten Kombinationen kaum vorkommen.

Zwei Kombinationen, hier in fettem Druck wiedergegeben, treten gleichzahlreich auf. Die obere (14_{II}) ist identisch mit der reinen

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1) Bei dem F_1-Bastard sind außer $7_{II}+7_{I}$ oft 1–2 Trivalente zu bemerken, wie schon in der Einleitung erwähnt wurde. Demnach müßten 1–2 semihomologe Chromosomen zwischen C- und D-Genom vorhanden sein. Durch Annahme eines „Crossing-over“ zwischen den semihomologen Chromosomen, das von Kostoff (1936) sowie von Kihara und Nishiyama (1937) festgestellt wurde, ließe sich ebenfalls die Schwankung der Chromosomenzahl in der Nachkommenschaft von Nr. 235 gut verstehen, ganz ebenso wie bei der oben erwähnten Translokation. Falls man nämlich annimmt, daß ein kleines Mittelstück zwischen den 2 Chromosomen ab und cd miteinander übereinstimmt, könnten wir ein neues Chromosom bc erhalten.

Schema I. Mögliche Kombinationen in der Nachkommenschaft von

$12_{II}+1_{III}\left(\begin{smallmatrix}ab & & cd\\ & \diagdown\ \diagup & \\ & bc & \end{smallmatrix}\right)$

♀ \ ♂	12+ab+cd zahlreich	12+bc+cd oft	12+ab+bc selten
12+ab+cd zahlreich	14_{II} $= 12_{II} + \begin{smallmatrix}ab\\ \|\|\\ ab\end{smallmatrix} + \begin{smallmatrix}cd\\ \|\|\\ cd\end{smallmatrix}$	$1_{IV}+12_{II}$, 14_{II} $= 12_{II} + \begin{smallmatrix}ab & & cd\\ \| & \diagup & \|\|\\ bc & & cd\end{smallmatrix}$	$1_{IV}+12_{II}$, (14_{II}) $13_{II}+2_{I}$ $= 12_{II} + \begin{smallmatrix}ab & & bc\\ \|\| & \diagup & \|\\ ab & & cd\end{smallmatrix}$
12+bc+cd oft	$1_{IV}+12_{II}$, 14_{II} $= 12_{II} + \begin{smallmatrix}ab & & cd\\ \| & \diagup & \|\|\\ bc & & cd\end{smallmatrix}$	* 14_{II} $= 12_{II} + \begin{smallmatrix}bc\\ \|\|\\ bc\end{smallmatrix} + \begin{smallmatrix}cd\\ \|\|\\ cd\end{smallmatrix}$	$1_{IV}+12_{II}$, $13_{II}+2_{I}$ $= 12_{II} + ab \diagup \begin{smallmatrix}bc\\ \|\|\\ bc\end{smallmatrix} \diagup cd$
12+ab+bc selten	$1_{IV}+12_{II}$, $13_{II}+2_{I}$, (14_{II}) $= 12_{II} + \begin{smallmatrix}ab & & bc\\ \|\| & \diagup & \|\\ ab & & cd\end{smallmatrix}$	$1_{IV}+12_{II}$, $13_{II}+2_{I}$ $= 12_{II} + ab \diagup \begin{smallmatrix}bc\\ \|\|\\ bc\end{smallmatrix} \diagup cd$	* 14_{II} $= 12_{II} + \begin{smallmatrix}ab\\ \|\|\\ ab\end{smallmatrix} + \begin{smallmatrix}bc\\ \|\|\\ bc\end{smallmatrix}$
12+bc zahlreich	$1_{III}+12_{II}$, $13_{II}+1_{I}$ $= 12_{II} + \begin{smallmatrix}ab & & cd\\ & \diagdown\ \diagup & \\ & bc & \end{smallmatrix}$	* $13_{II}+1_{I}$, ($1_{III}+12_{II}$) $= 12_{II} + \begin{smallmatrix}bc\\ \|\|\\ bc\end{smallmatrix} \diagdown cd$	* $13_{II}+1_{I}$, $1_{III}+12_{II}$ $= 12_{II} + ab \diagup \begin{smallmatrix}bc\\ \|\|\\ bc\end{smallmatrix}$
12+ab oft	$13_{II}+1_{I}$ $= 12_{II} + \begin{smallmatrix}ab\\ \|\|\\ ab\end{smallmatrix} + cd$	$1_{III}+12_{II}$, $13_{II}+1_{I}$ $= 12_{II} + \begin{smallmatrix}ab & & cd\\ & \diagdown\ \diagup & \\ & bc & \end{smallmatrix}$	* $13_{II}+1_{I}$, $1_{III}+12_{II}$ $= 12_{II} + \begin{smallmatrix}ab\\ \|\|\\ ab\end{smallmatrix} \diagdown bc$
12+cd selten	$13_{II}+1_{I}$ $= 12_{II} + \begin{smallmatrix}cd\\ \|\|\\ cd\end{smallmatrix} + ab$	* $13_{II}+1_{I}$, ($1_{III}+12_{II}$) $= 12_{II} + bc \diagup \begin{smallmatrix}cd\\ \|\|\\ cd\end{smallmatrix}$	$1_{III}+12_{II}$, $13_{II}+1_{I}$ $= 12_{II} + \begin{smallmatrix}ab & & cd\\ & \diagdown\ \diagup & \\ & bc & \end{smallmatrix}$
12+ab+bc+cd sehr selten	$14_{II}+1_{I}$, $1_{III}+13_{II}$ $= 12_{II} + \begin{smallmatrix}cd\\ \|\|\\ cd\end{smallmatrix} + \begin{smallmatrix}ab\\ \|\|\\ ab\end{smallmatrix} \diagdown bc$	$14_{II}+1_{I}$, $1_{III}+13_{II}$ $= 12_{II} + \begin{smallmatrix}cd\\ \|\|\\ cd\end{smallmatrix} + ab \diagup \begin{smallmatrix}bc\\ \|\|\\ bc\end{smallmatrix}$	$14_{II}+1_{I}$, ($1_{III}+13_{II}$) $= 12_{II} + \begin{smallmatrix}ab\\ \|\|\\ ab\end{smallmatrix} + \begin{smallmatrix}bc\\ \|\|\\ bc\end{smallmatrix} \diagdown cd$

Art *Ae. cylindrica* und die untere ($1_{III}+12_{II}$) gleich Nr. 235. In der Tat wurden 17 Pflanzen mit 14_{II} und 16 mit $1_{III}+12_{II}$ erhalten (Tab. 6 und 8). Ihr Verhältnis ist demnach ungefähr 1:1. Zweitens müssen, wie sich aus dem Schema ergibt, die Pflanzen mit $1_{IV}+12_{II}$ bzw. $13_{II}+1_{I}$ oft auftreten. In Wirklichkeit wurden 10 bzw. 7 Pflanzen mit den entsprechenden Kombinationen beobachtet.

In Schema I wird meist ein Tetrapartites durch die Kombination (bc + cd) + (ab + cd), selten durch (ab + bc) + (ab + cd) gebildet. Dagegen kommt fast nie die Kombination (ab + bc) + (bc + cd) vor. Das Tetrapartite $\begin{pmatrix} ab & & cd \\ | & / & \| \\ bc & & cd \end{pmatrix}$ wird oft in 2 Bivalente $\begin{pmatrix} ab & & cd \\ | & + & \| \\ bc & & cd \end{pmatrix}$ aufgelöst, während aus dem Komplex $\begin{pmatrix} ab & & bc \\ \| & / & | \\ ab & & cd \end{pmatrix}$ manchmal $1_{II} + 2_{I}$ $\begin{pmatrix} ab & & \\ \| & + bc + cd & \\ ab & & \end{pmatrix}$ oder selten $2_{II}\begin{pmatrix} ab & & bc \\ \| & + & | \\ ab & & cd \end{pmatrix}$ entsteht. Ebenfalls wird bei der Kombination $13_{II}+1_{I}$ im oberen Falle das Univalente durch das Chromosom cd und im unteren durch ab gebildet.

Schema II. Mögliche Kombinationen in der Nachkommenschaft von $12_{II}+1_{IV}\begin{pmatrix} ab & & cd \\ | & / & \| \\ bc & & cd \end{pmatrix}$

♀ \ ♂	12+ab+cd	12+bc+cd
12+ab+cd	14_{II} $= 12_{II}+ \begin{matrix} ab \\ \| \\ ab \end{matrix} + \begin{matrix} cd \\ \| \\ cd \end{matrix}$	$1_{IV}+12_{II}$, 14_{II} $= 12_{II}+ \begin{matrix} ab & & cd \\ | & / & \| \\ bc & & cd \end{matrix}$
12+bc+cd	$1_{IV}+12_{II}$, 14_{II} $= 12_{II}+ \begin{matrix} ab & & cd \\ | & / & \| \\ bc & & cd \end{matrix}$	* 14_{II} $= 12_{II}+ \begin{matrix} bc \\ \| \\ bc \end{matrix} + \begin{matrix} cd \\ \| \\ cd \end{matrix}$

Wir wollen weiter durch die gleiche Annahme die Chromosomenzahlschwankung in den Folgegenerationen erklären, wofür die Schemata II und III einzusehen sind. Schema II bringt die möglichen Kombinationen bei der Nachkommenschaft der Pflanzen mit $12_{II}+1_{IV}$ $\begin{pmatrix} ab & & cd \\ | & / & \| \\ bc & & cd \end{pmatrix}$, wo das Zahlenverhältnis von 14_{II} und $1_{IV}+12_{II}$ ungefähr $1:2>$ ist (Tab. 9). In diesem Falle müssten die Pollenkörner mit 12+ab+cd bei der Befruchtung etwas tüchtiger sein, als die mit 12+bc+cd. Bei der Rückkreuzung $12_{II}+1_{IV}$ $\begin{pmatrix} ab & & cd \\ | & / & \| \\ bc & & cd \end{pmatrix}$ $\times$ *Ae. cylindrica* ist auch das entsprechende Verhältnis 1 : 1 (Tab. 10). Die Kombinationen der Nachkommen der 29-chromosomigen Pflanze $\left(12_{II} + \begin{matrix} cd \\ \| \\ cd \end{matrix} + \begin{matrix} ab & \\ \| & \diagdown bc \\ ab & \end{matrix}\right)$ sind in Schema III zusammengestellt (Tab. 11).

Schema III. Mögliche Kombinationen in der Nachkommenschaft von $14_{II}+1_{I}\left(= 12_{II}+ \begin{matrix} cd \\ \| \\ cd \end{matrix} + \begin{matrix} ab & \\ \| & \diagdown bc \\ ab & \end{matrix}\right)$

♀ \ ♂	12+ab+cd	12+ab+bc+cd
12+ab+cd	14_{II} $= 12_{II}+ \begin{matrix} ab \\ \| \\ ab \end{matrix} + \begin{matrix} cd \\ \| \\ cd \end{matrix}$	$14_{II}+1_{I}$, $1_{III}+13_{II}$ $= 12_{II}+\begin{matrix} cd \\ \| \\ cd \end{matrix}+\begin{matrix} ab & \\ \| & \diagdown bc \\ ab & \end{matrix}$
12+ab+bc+cd	$14_{II}+1_{I}$, $1_{III}+13_{II}$ $= 12_{II}+\begin{matrix} cd \\ \| \\ cd \end{matrix}+\begin{matrix} ab & \\ \| & \diagdown bc \\ ab & \end{matrix}$	15_{II} $= 12_{II}+\begin{matrix} ab \\ \| \\ ab \end{matrix}+\begin{matrix} bc \\ \| \\ bc \end{matrix}+\begin{matrix} cd \\ \| \\ cd \end{matrix}$

Zusammenfassung der Ergebnisse

1) Der Bastard *Ae. caudata* L. ($n=7$)$\times$ *Ae. cylindrica* HOST ($n=14$) hat $7_{II}+7_{I}$ bei der Reifungsteilung der PMZ. Das gemeinsame Genom beider Arten ist C, weil mit der Dinkelreihe von *Triticum* (ABD) *Ae. caudata* kein homologes Genom hat, während *Ae. cylindrica* ein homologes (D) aufweist.

2) Die Beobachtungen bei der Rückkreuzung $F_1\times$*Ae. caudata* haben folgendes ergeben. Das *Caudata*-Genom (C_{caud}) ist völlig austauschbar mit dem C_{cyl}-Genom von *Ae. cylindrica.* Die Rekombination zwischen beiden Genomen hat keinen Einfluß auf die Fruchtbarkeit. Auch die morphologischen Eigenschaften lassen einfach die MENDELsche Vererbung erkennen, was auf der Rekombination der betreffenden Chromosomen beruht.

3) Auf Grund der Rückkreuzung $F_1 \times$ *Ae. cylindrica* war eine Pflanze 27-chromosomig. Die Erklärung für die Chromosomenzahlschwankung bei den Folgegenerationen dieser Pflanze wurde gerade oben gegeben, wonach diese Schwankung eben auf der reziproken Translokation zwischen 2 Chromosomen des F_1-Bastards beruht. Diese Translokation kann entweder zwischen 2 Chromosomen des gleichen Genoms (C oder D) oder zwischen dem C- und D-Chromosom stattfinden.

Zum Schluß möchten wir hinzufügen, daß wir der Japanischen Gesellschaft zur Förderung der Zytologie für die gewährte finanzielle Unterstützung zur Ausführung dieser Arbeit zu großem Dank verpflichtet sind.

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Cytological Studies of Sugar Cane. I
Observations on some POJ varieties*

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Introduction

Cytological studies of the genus *Saccharum* were first undertaken by FRANK (1910) and KUWADA (1915, 1919), and since then many authors in various countries have been engaged in this work. However, most of our knowledge of cytology of sugar cane is due to BREMER (1922–34).

The present paper deals with the reduction division of PMC of 5 POJ varieties (2725 POJ, 2722 POJ, 2878 POJ, 2883 POJ and 2364 POJ) and one variety belonging to *S. spontaneum*. Special attention was paid to the number and behaviour of chromosomes during the maturation divisions of PMC.

Material and Methods

2725 POJ, 2722 POJ, 2878 POJ, 2883 POJ and 2364 POJ, which had been cultivated in the experimental field of Ensuikō Sugar Manufacturing Co. Ltd. (Sin'yei, Taiwan), were used for this study. The clones of these POJ varieties are given in Table 1. A variety belonging to *S. spontaneum* used in the present investigation was collected at Karenkō, East Formosa, some years ago. All the materials used were fixed in CARNOY's fluid for about 2 or 3 hours and stained with aceto-carmine.

Many smear preparations were made for this observation. However, rarely excellent plates of I. metaphase where even a single doubtful chromosome element was never present, were obtained. Analysis of chromosome conjugation was made only from such figures. Accordingly the number of PMCs used for POJ varieties was not large, being 10–20.

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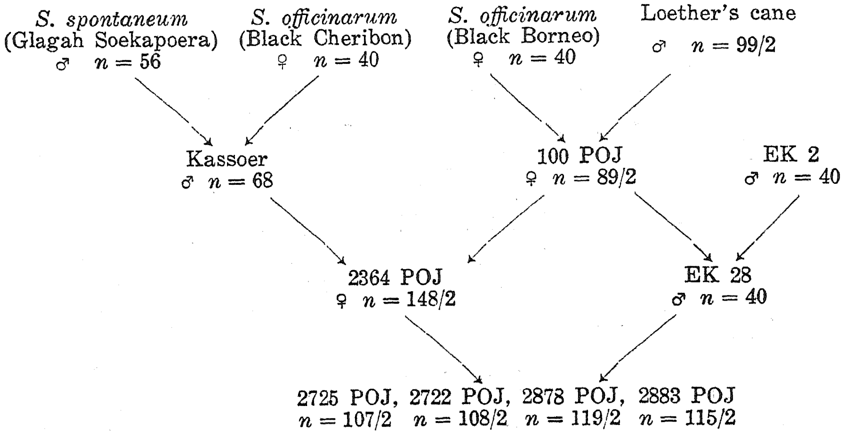
Table 1. The pedigree of POJ varieties.



Observations

1. 2725 POJ.

2725 POJ showed commonly $50_{II} + 7_{I}$ (1fr.), or $49_{II} + 9_{I}$ (includ. 1fr.) and in some cases $3_{IV} + 44_{II} + 7_{I}$ (1fr,), $3_{IV} + 1_{III} + 43_{II} + 6_{I}$ (1fr.), or $2_{IV} + 1_{III} + 44_{II} + 8_{I}$ (1fr.) at the I. metaphase of PMC (Tab. 2, Figs. 1–3). Hence, the total chromosome number in this variety is 107. The number of the univalents are 6—*ca.*14(1fr.). It is remarkable that in this variety there is always one fragment chromosome.

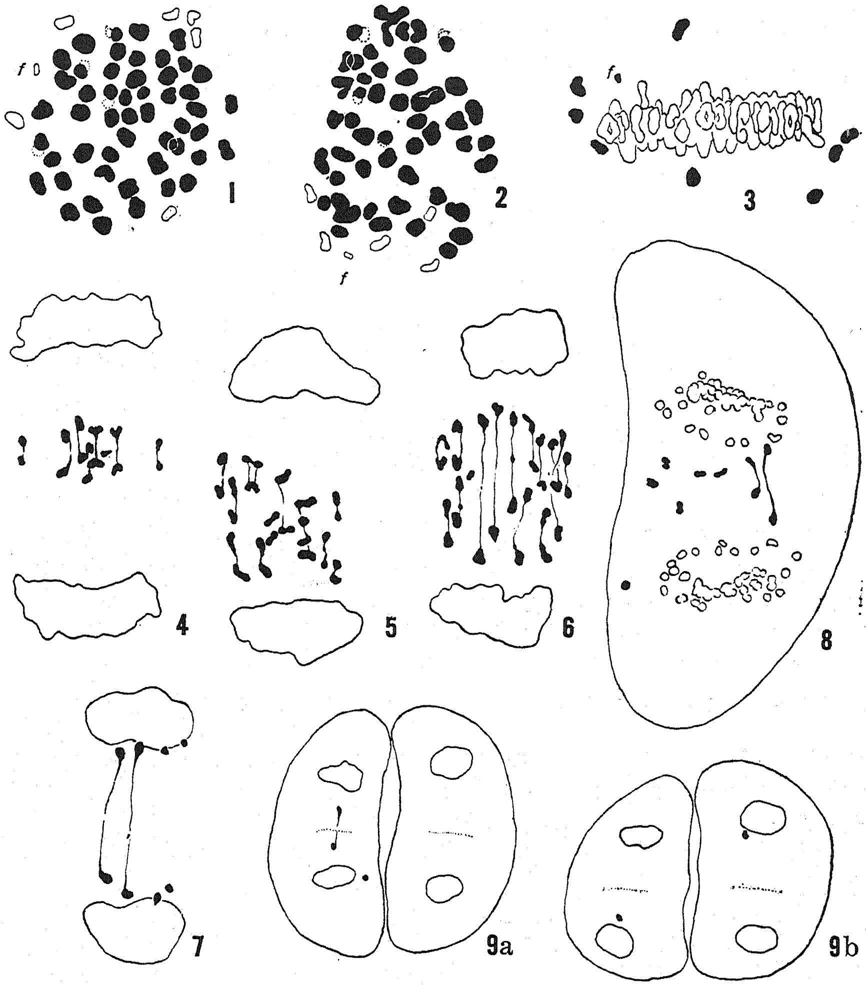
In general, at the I. anaphase the univalents divide longitudinally and at the II. anaphase they are distributed to different poles at random, but in some cases the univalents, which have failed to divide at the I. anaphase, separate longitudinally at the II. anaphase (Fig. 8).

When the univalents are about to divide longitudinally at the I. anaphase, the divided halves of the univalents often draw one or two chromatin threads as if they were the chromosome bridges (Figs. 4–6.). These univalents often lag behind the daughter nuclei and form one or two small nuclei (Fig. 7). At the II. telophase and at the

Tab'e 2. Number of bivalents and univalents

Varieties	Conjugation	n	No. of univalents
2725 POJ	$50_{II} + 7_{I}$ (1fr.) $49_{II} + 9_{I}$ (1fr.)	107/2	6 (1fr.)—14 (1fr.)
2722 POJ	$50_{II} + 8_{I}$ (1fr.) $49_{II} + 10_{I}$ (1fr.)	108/2	8 (1fr.)—14 (1fr.)
2878 POJ	$57_{II} + 5_{I}$ $56_{II} + 7_{I}$	119/2	4—13
2883 POJ	$55_{II} + 5_{I}$ $54_{II} + 7_{I}$	115/2	5—14
2364 POJ	$67_{I} + 14_{I}$	148/2	6—16
S. spontaneum alas Karenkō	48_{II}	48	0

pollen tetrad stage, the lagging monad chromosomes derived from the univalents often form one or more small nuclei (Fig. 9a, b).



Figs. 1–9. Reduction divisions of 2725 POJ. **1.** Polar view of the I. metaphase. $50_{II}+7_{I}$ (1fr.) = 107. ×1815. **2.** *Ditto.* $3_{IV}+1_{III}+43_{II}+6_{I}$ (1fr.) = 107. ×1815. **3.** Side view of the I. metahase. 8_{I} and 1 fragment (f.) are clearly observed. ×1815. **4–6.** The I. anaphase. The halves of univalents are connected with chromatin threads, when univalents are about to separate longitudinally. ×1815. **7.** The I. telophase. Lagging monads derived from univalents. ×1815. **8.** The II. anaphase. The univalents, which presumably failed to divided at the I. anaphase, separate longitudinally at the II. anaphase. ×1815. **9a, b.** The II. telophase. Lagging monads and small nuclei. ×880.

2. 2722 POJ, 2878 POJ, 2883 POJ and 2364 POJ.

The number of bivalents and univalents at the I. metaphase of PMC in these 4 varieties is shown in Table 2. Irregularities in

the reduction division of these varieties were similar to those of 2725 POJ.

2722 POJ: 2722 POJ showed commonly $50_{\rm II} + 8_{\rm I}$ (1fr.) or $49_{\rm II} + 10_{\rm I}$ (1fr.), namely $n = 108/2$ (Fig. 10). This variety always has one fragment, too. It seems that this fragment chromosome may have the same origin as the one found in 2725 POJ, because both 2722 POJ and 2725 POJ have been derived from the same cross, (2364 POJ × EK 28). Univalents varied from 8 to *ca.* 14 including one fragment.

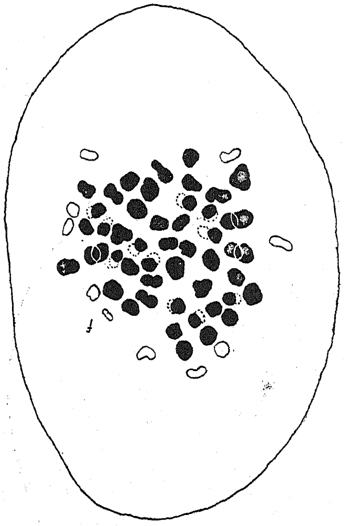
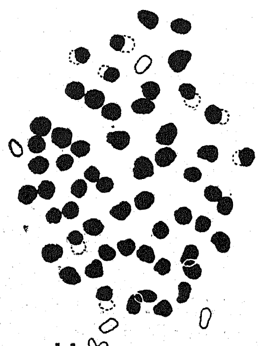


Fig. 10. Polar view of the I. metaphase in 2722 POJ. $49_{\rm II}+10_{\rm I}$ (1fr.) = 108. ×1815.

2878 POJ: 2878 POJ showed commonly $57_{\rm II} + 5_{\rm I} = 119$ or $56_{\rm II} + 7_{\rm I} = 119$ (Figs. 11–12). In some cases tetravalents (0–1) and trivalents (0–1) were found. Univalents varied from *ca.* 4 to 13. In some preparations, the author observed 2–5 bivalents associated closely with one another just like so-called secondary associations.

2883 POJ: In this variety $54_{\rm II}+7_{\rm I}$ or $55_{\rm II} + 5_{\rm I}$ (namely $n = 115/2$), was usually observed (Fig. 13). Univalents varied from 5 to *ca.* 14.

2364 POJ: The chromosome number was found to be $n = 148/2$, and the chromosome conjugation was in most cases $67_{\rm II}+14_{\rm I}$ (Figs. 14–16.). Univalents varied from 6 to *ca.* 16.



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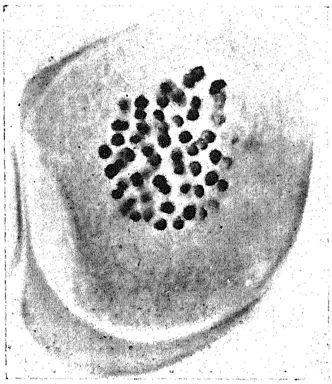

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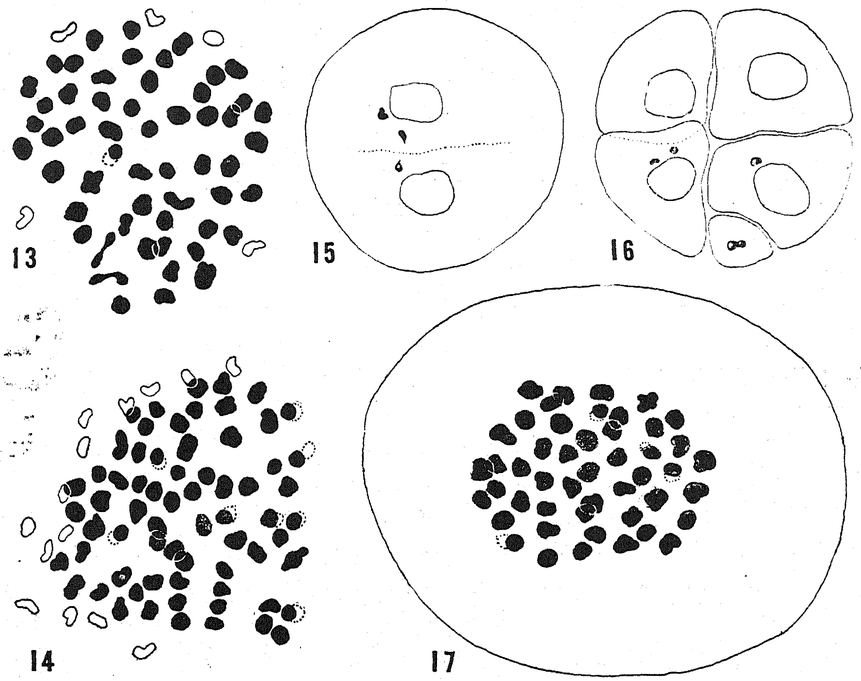
Figs. 11–12. **11.** Polar view of the I. metaphase in 2878 POJ. $57+_{\rm II}+5_{\rm I} = 119$. 2–5 bivalents associate closely with one another. ×1815. **12.** Photomicrograph, polar view of I. metaphase in 2878 POJ. × ca. 950.

3. *S. spontaneum* L. alas Karenkō.

This variety showed invariably $48_{\rm II}$ at the I. metaphase of PMC (Fig. 17). The division was quite regular throughout the meiotic stage and almost all pollen grains were quite normal.



Figs. 13–17. 13. Polar view of the I. metaphase in 2883 POJ. $55_{II}+5_{I}=115$. $\times 1815$. 14–16. Reduction divisions of 2364 POJ. 14. Polar view of the I. metaphase. $67_{II}+14_{I}=148$. $\times 1815$. 15. The I. telophase, showing lagging monads. $\times 880$. 16. Pollen tetrads. 5 cells with small nuclei are shown. $\times 880$. 17. The I. metaphase of *S. spontaneum* L. alas Karenkō with 48_{II}. $\times 1815$.

Discussion

2725 POJ: The total chromosome number of 2725 POJ in one PMC determined from the I. anaphase, was found to be 106–107 by BREMER (1928). YAMASHITA (1937) and MORIYA (1940) observed 107 chromosomes in the root tip cells. As the present author's counts also agree with those of the previous authors, the diploid chromosome number in this variety is probably 107 including one fragment, which has not hitherto been mentioned. The number of bivalents and univalents of this variety is 50 and 7 (1fr.), or, in most cases, 49 and 9 (1fr.) respectively. BREMER did not determine the chromosome number in PMC.

2722 POJ: According to BREMER (1928), the total chromosome number at the anaphase of 2722 POJ is 108. 47 dyads on each daughter plate and 14 univalents on the equator were shown in his figure. The author's observations on the I. metaphase show that this variety has commonly either $50_{II}+8_{I}$ (1fr.) or $49_{II}+10_{I}$ (1fr.), namely the diploid number is 108, and the univalents vary from 8

to ca. 14. The diploid chromosome number calculated by the author agrees with that of BREMER. One fragment observed in this variety is probably the same one as that of 2725 POJ.

2878 POJ: BREMER (1928, 1931) reported that 2878 POJ has 119–120 chromosomes at the I. anaphase and that in root tip cells 119–121 chromosomes are observed. According to YAMASHITA (1937), this variety has 119 in somatic mitosis. The total number which the author counted at the I. metaphase of this variety, agreed with YAMASHITA's result. After BREMER, this variety has 56 gemini and 7 univalents inferred from the anaphase figure. The estimation is verified by the author's counts at the metaphase, where either $57_{II} + 5_{I}$ or $56_{II} + 7_{I}$ is detected in most cases and univalents vary from *ca.* 4 to 13.

There was clear indication of secondary associations, as JANAKI-AMMAL (1936) observed in *S. spontaneum*. Detailed examinations could not be made owing to the lack of preparations. It is desirable to elucidate the nature of this phenomenon in these POJ varieties.

2883 POJ: YAMASHITA (1937) observed *ca.* 115 in somatic cells. 114–115 chromosomes were counted by BREMER (1928) in the meiotic division. From the figures of I. anaphase, he calculated the number of bivalents (37–38) and univalents (*ca.* 40). Such figures could not be found in the I. metaphase by the author. There are usually $54_{II} + 7_{I}$ or $55_{II} + 5_{I}$ in 2883 POJ. Hence the diploid number is 115. Univalents varied from 5 to *ca.* 14.

2364 POJ: 2364 POJ has most commonly $67_{II} + 14_{I}$, namely 148 chromosomes, and the univalents vary from 6 to *ca.* 16, while after BREMER (1928) this variety has 60 gemini and 28 univalents, (namely, the diploid number is 148), inferred from the I. anaphase figure, and the univalents vary from 16 to 26. 148 diploid chromosomes were really found in root tip cells by BREMER (1931).

Discrepancy between the conjugation relationships in 2883 POJ and 2364 POJ observed by BREMER and by the present author is perhaps due to environmental difference between Java and Formosa.

S. spontaneum L. alas Karenkō: It is reported that in *S. spontaneum* there are many varieties having different chromosome number, namely $2n = 48$, 56, 64, 72, 80, 96, *ca.*112, 124, etc. (BREMER, 1934; JANAKI–AMMAL, 1936; JANAKI-AMMAL & SINGH 1936; SANTOS, 1937)

BREMER (1934) found that *S. spontaneum* Madaly I. (from Burma) and *spontaneum* clones of Middle Celebes, which bear the local name of Biroh, have $2n = 96$. JANAKI–AMMAL & SINGH (1936) reported also that *S. spontaneum* L. Burma form had the same chromosome number.

MORIYA (1940) reported in his recent paper that three *spontaneum* varieties having $n = 48$, 56 and 104/2 were found. After him Kanshogaya (*S. spontaneum* L. subsp. *indicum* HACKEL var. *Roxburghii* HONDA) collected in Tainan, South Formosa, has 48 chromosomes at the I. metaphase of PMC.

Since *S. spontaneum* L. alas Karenkō examined by the author, has 48_{II} at the I. metaphase of PMC, it seems that this variety is closely related to the above mentioned varieties.

Summary

1. The number of bivalents and univalents at the I. metaphase of PMC in 2725 POJ, 2722 POJ, 2878 POJ, 2883 POJ and 2364 POJ has been examined (Table 2).

2. Both 2725 POJ and 2722 POJ have one fragment each. These two fragments have perhaps the same origin, as the two varieties have been derived from the same cross (2364 POJ × EK 28).

3. In some preparations of 2878 POJ, it has been observed that 2–5 bivalents are closely arranged, showing so-called secondary associations.

4. *S. spontaneum* L. alas Karenkō, which has been collected at Karenkō, East Formosa, shows 48_{II} at the I. metaphase of PMC.

Acknowledgment

In conclusion, I wish to express my sincere thanks to Prof. H. KIHARA and Dr. I. NISHIYAMA for their kind direction throughout the course of this investigation. Thanks are also due to Dr. A. GONDO and Dr. K. YAMASHITA for their invaluable advice and criticism.

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Diploid-Bud Formation in a Haploid *Oryza* with Some Remarks on the Behaviour of Nucleolus in Mitosis[1)2)]

By

Kono Yasui

(With 19 Text Figures)

Received January 28, 1941

Introduction

Since the haplosporophyte of *Datura* was discovered and the diplosporophyte was synthetically formed by BLAKESLEE et alii (1922, 1923) the problem of homology of chromosome complements has attracted special attention of the workers on cytogenetics. In the genetical studies the heterogenity of the chromosome complements of the material may impair the validity of the results of experiments even when we are concerned with only one genic character, but the genetical as well as physico-chemical test of the absolute homology of chromosomes in diplosporophyte is hardly possible; consequently the formation of homologous diploid complements by the simple duplication of the chromosome complement of a haploid plant is desirable, as such an autodiploid will serve as a most reliable material for genetic studies.

There are two ways of obtaining absolutely duplicated chromosome complements; namely through the seed from haplosporophyte and through bud variation, the latter being preferable, because the vegetative duplication of the chromosomes is simpler than the former.

The writer's present case concerns a diplosorophyte of the rice plant derived through a bud variation in a stock of the haplosporophyte.

Material and Method

Several years ago a stock of a sterile rice plant was kindly put at my disposal from the Kōnosu Branch of the Imp. Agric. Exper. Station of the Dept. of Agriculture & Forestry, with the information that it was a triplosporophyte. Since then it has been cultivated in a pot which was kept in the green-house of the Botanical Institute, Faculty of Science, Tokyo Imperial University, throughout the year except in the summer when it was kept outdoors. The plant propagated vegetatively with nothing particular to remark until 1939, the

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1) Contributions from the Divisions of Plant Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 278.

2) Aided by a grant from the Japan Society for the Advancement of Cytology.

panicles being always sterile. In the summer of 1939 which was extremely hot some of the culms grew with unusual vigour and the panicles were partially fertile, while in some others the panicles were completely sterile (Fig. 1). Those culms were separated as three different clones. For the karyological studies the root-tips were gathered from each clone and fixed with NAVASHIN's fluid. The paraffin sections were stained after NEWTON's gentian violet method and also with HEIDENHAIN's iron-alum haematoxylin, the former being found more suitable. The photomicrographs for the chromosome study were taken with the aid of a ZEISS objective H. I. ×100 and LEITZ peripl. oc. ×10, and for the topographical study of tissue the ZEISS objective C and LEITZ peripl. oc. ×8 were used. The measurements of the cell and nuclear sizes were made after the camera drawings, showing the maximum diameters and made with the aid of ZEISS H. I. ×100 and K oc. ×15.

Fig. 1. Rice plants propagated from a sterile stock; *a*, original sterile stock; *b*, almost sterile panicles; *c*, fertile panicles. ca.1/7.

Observation

Original plant. The plant is dwarfish, being about 60 cm. high (Figs. 1–3,*a*). New buds were formed one by one from the old stock and flowered in the pot even in the winter season in the green house, though the flowers were abortive. The roots were thin, being 0.36 mm or less in the diameter of the cross section (Fig. 4). The chromosome number in the root cells was found to be 12 (Fig. 13) which is the known haploid number in the ordinary rice plants (KUWADA 1909, etc.), but not 36 as we might have expected for this stock. In fact this stock was a haplosporophyte.

The fertile clone. The culm was about 1 m high, about one and half times the original plant, and looked more vigorous than the latter (Figs. 1 and 2, *c*). The panicle nodded (Fig. 1, *c*) when the grains grew ripe, unlike the haploid panicles (Fig. 1, *a*) which stood upright even when they had dried up after flowering. The roots were thicker than those of the original plant, the diameter of the cross sections measuring more than 0.46 mm generally. The chromosome number in the root-tip cells was 24, which is the twice that of the original stock and the same with that of the ordinary rice plants.

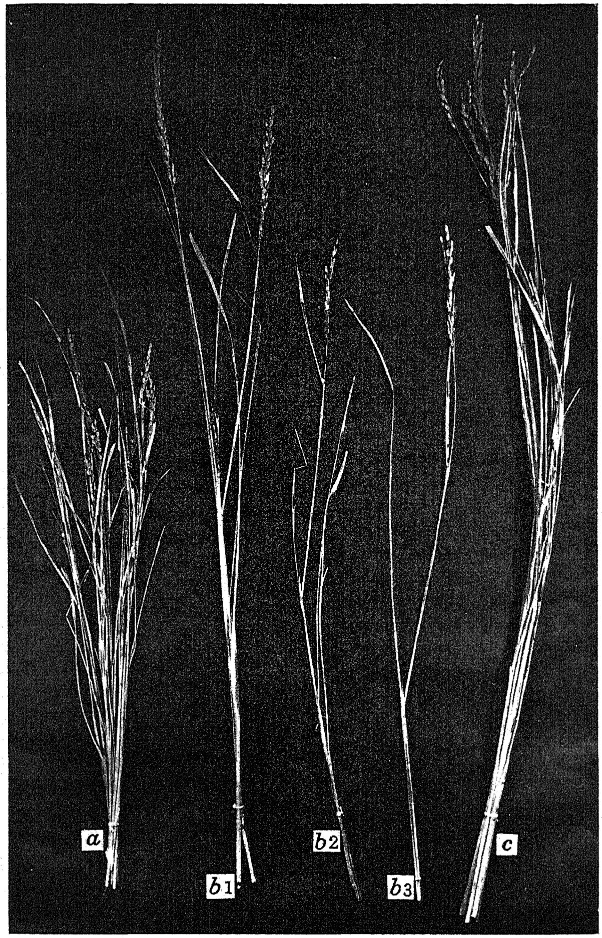
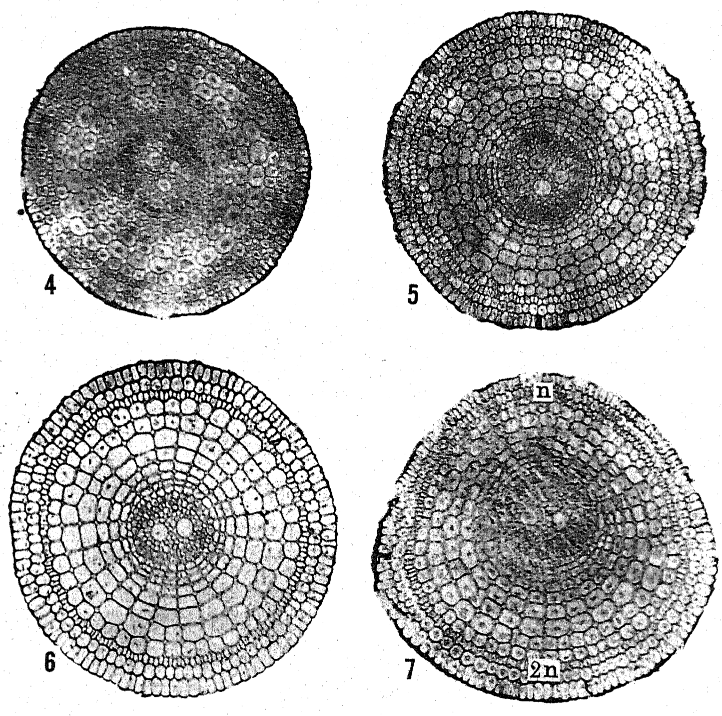


Fig. 2. Culms with panicles. *a*, original sterile stock; *b*, semisterile clone; b_1, almost abortive, b_2 and b_3, fertile grains mixed with aborted flowers; *c*, fertile clone. ca. ×1/7.

The semi-sterile clone (chimera). The height resembled that of the fertile clone, but the panicles had grains mixed with some aborted flowers (Figs. 2 and 3, *b*). The ratio of the fertile grains to the aborted flowers varied greatly in the different panicles, ranging from almost fertile to almost sterile. Some roots in cross sections consisted entirely of the haploid cells (Figs. 5 and 10), and some entirely of diploid cells, (Figs. 6, 11) while one of them was a sectorial chimera (Figs. 7 and 12). The thickness of the roots did not differ much in cases where they consisted entirely of the diploid or haploid cells; but in such cases the number of the cells in the same dimentions of root were different, larger in the haploid and smaller in the diploid, consequently the sizes of the cells were

Fig. 3. The panicles. *a*, from original haplosporophyte; *b*, from semisterile clone (chimera), *c*, from fertile clone. ca. ×3/10.



Figs. 4-7. Photomicrographs of the cross sections of the roots. 4, original haploid; 5–7, semisterile clone; 5, haploid root; 6, diploid root; 7, chimerical root. ca. ×115.

contrary to the numbers (Figs. 5, 6, 10 and 11). In the sectorial chimera the dimension of the cells and also of the tissue in the haploid part was smaller than that in the diploid part (Figs. 7 and 12). Table 1 shows both the radial and tangential average diameters of the cortical cells in the cross section of the chimerical root.

Table 1. The radial and tangential diameters of the cortical cells in the 3rd-8th cell layers, counting from the epidermis of the cross section of the chimerical root.

Cell layer	3rd		4th		5th		6th		7th		8th	
Av. cell diameter	Rad.	Tang.	Rad.	Tang.	Rad.	Tang.	Rad.	Tang.	Rad.	Tang.	Rad.	Tang.
Diploid	18.0	17.5	23.2	26.7	24.0	30.0	20.1	30.6	15.5	25.6	11.9	20.4
Haploid	14.5	15.0	16.9	22.6	13.7	18.8	12.2	29.9	9.4	16.6	7.5	5.7

We could not measure the actual volume of the cells in the chimerical root, so that the ratio of the volumes of the diploid cells to the haploid ones was measured from the average areas of the cross section of the respective cells in the chimerical root.

The frequency polygons of the radial and tangential diameters in micron of the cross sections of the cortical cells in general of the chimerical root are shown with their constants in figure 8.

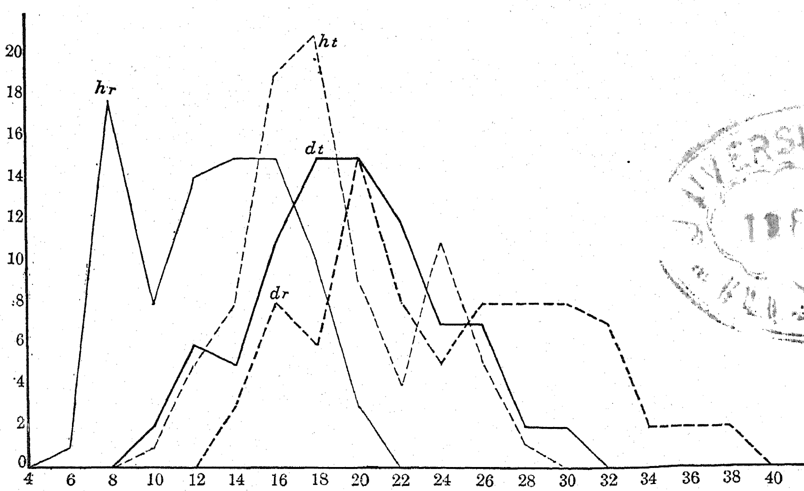
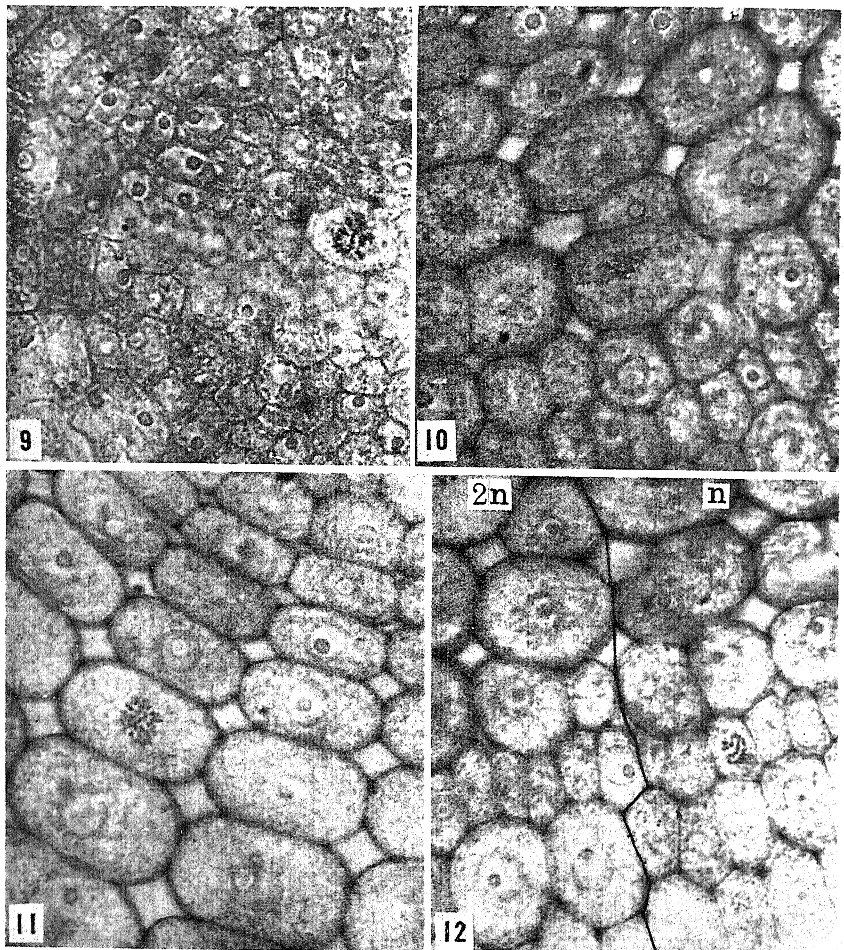


Fig. 8. The frequency polygon of the radial and tangential diameters of the cross section of the cortical cells of the chimerical root. N = 84; *hr*, radial diameter of the haploid cells; *ht*, tangential diameter of the latter; *dr* and *dt*, radial and tangential diameters of the diploid cells respectively; $M_{hr} = 12.95$; $\sigma_{hr} = 3.66$; $m_{hr} = 0.4$; $m_{\sigma hr} = 0.26$; $M_{ht} = 21.5$; $\sigma_{ht} = 4.00$; $m_{ht} = 0.47$; $m_{\sigma ht} = 0.30$; $M_{dr} = 19.41$; $\sigma_{dr} = 4.55$; $m_{dr} = 0.5$; $m_{\sigma dr} = 0.35$; $M_{dt} = 22.95$; $\sigma_{dt} = 5.46$; $m_{dt} = 0.6$; $m_{\sigma dt} = 0.42$.

The ratio of the square root of the average area of the diploid cells to that of the haploid cells is 1.26, that is near to $\sqrt[3]{2}$; consequently we can consider that the diploid cells in this chimerical root have twice the volume of the haploid cells (Table 2).

Table 2. A comparison of the volumes of the diploid and haploid cells.

	Average area of the cross section	Square root of the average area	Ratio (1) to (2)
Diploid	445.46	21.1 (1)	1.26
Haploid	278.43	16.7 (2)	1.00



Figs. 9–12. Parts of the cross sections of the roots of the original haploid and semisterile clone. 9, a part of the stele of the original haploid; 10–12, cortical tissues from semisterile clone; 10, haploid root; 11, diploid root; 12, chimerical root. To the left of the dark line of demarkation, diploid tissue; to the right, haploid tissue. ca. ×1000.

Somatic nucleus and chromosome complements in the haploid cells. In the interkinetic nucleus the chromosomes are disentangled completely and spread over in the nuclear cavity as fine homogeneous threads which stain faintly with chromatic stains. The nucleolus occupied the greater part of the nuclear cavity and in the living state a thin but strong refractive layer was observed between the nucleolus and the karyolymph, but no such conspicuous space ("Hof") as we see in the fixed material (Figs. 9–12).

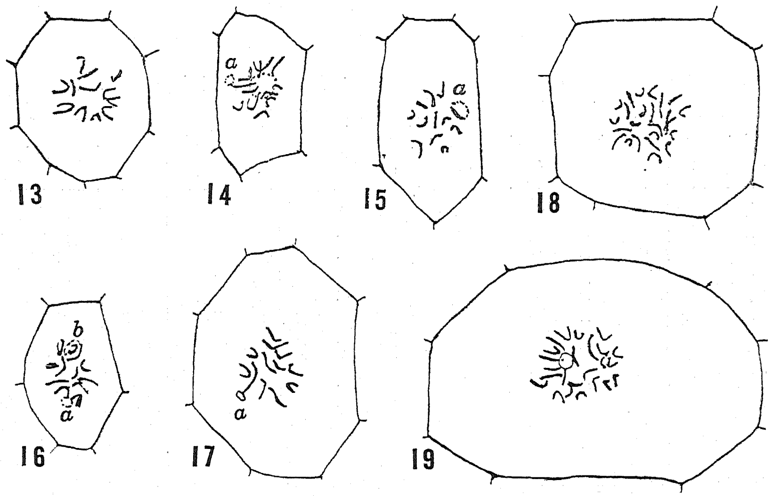


Fig. 13. A cell of a young vessel in a root of the original haplosporophyte. Figs. 14–17. Cortical haploid cells in the root of the semisterile clone. 14, several chromosomes are seen attached to a main nucleolus at the center of the mitotic figure; one end of a chromosome is seen attached to a nucleolar fragment (a), and the other end to the main body of the nucleolus; 15, so-called nuleolar chromosome attached to a nucleolar fragment (a); 16, a, two chromosomes attached to one and the same nucleolar fragment by one of their ends; b, the nucleolar chromosome attached to the periphery of a nucleolar fragment; 17, a long chromosome attached to a nucleolar framgent (a). Figs. 18, 19. Cortical diploid cells. 18, 24 chromosomes without nucleolar attachment; 19, 4 chromosomes in metaphase attached to a nucleolar fragment.

In the early prophase chromatin threads became distinct and most of them (probably all of them) came to attach themselves to the nucleolus and gradually became thicker and developed into chromosomes. In this stage the "Hof" around the nucleolus is invisible even in the fixed material, and the nucleolar substance seems to be pliant and sticky. The chromonemata attach to the nucleolus with special points where they are probably naked from the hyalonema (the matrix), namely with the constriction regions and the end of the chromosomes. Afterwards the chromosomes detach from the nucleolus one by one, some of them holding the fragments of the

nucleolus which simultaneously divides. In the early metaphase several chromosomes holding the fragments of the nucleolus were observed (Figs. 13–16), though they disappeared in the later metaphase, except one on which the nucleolus was attached until the beginning of the anaphase. The average diameters of the nucleus and of the nucleolus in the fixed material is 7.14μ and 2.68μ respectively.

Chromosome complement in the haploid nucleus. Due to the smallness of the chromosomes the accurate measurements of length were very difficult, so that the chromosome sizes in the following table are not decisive, though they are based on several measurements.

Table 3. Chromosome complement from a haploid nucleus.

No. of the chromosome	Relative length of the chromosomes	Ratio of the short arm of the chromosomes to the whole length	Remark
1	ca. 5.0	ca. 2/5	
2	,, 4.4	,, 1/2	
3	,, 4.1	,, 1/2	
4	,, 3.8	,, 1/2	
5	,, 3.7	,, 2/5	
6	,, 3.5	,, 2/5	
7	,, 3.1	,, 2/5	
8	,, 3.0	,, 1/3	
9	,, 2 9	,, 1/2	
10	,, 2.6	,, 1/6	SAT-chromosome
11*	,, 2.3	,, 2/5	
12	,, 2.0	,, 3/8	

* The 11th chromosome that which retains nucleolar fragment for the longest time.

From the data in the table we see that the our chromosome complement is somewhat different from that given by NANDI (1936).

Chromosome complements in the diploid cells. The detailed morphological comparison of the chromosome complements of the x- and $2x$-nuclei has not been made, but a gross classification of the chromosome in sizes and shapes in the $2x$-cells, especially the double occurrence of the so-called nuclear- and SAT-chromosomes in the $2x$-cells shows that the 24 chromosomes in the $2x$-cells were derived by the doubling of the chromosome complement of the x-nucleus.

The average diameter of the nucleus and of the nucleolus of the $2x$-cells in the fixed material is 8.21μ and 2.86μ respectively. The ratio of these diameters to those of the x-cells is 1.15 and 1.07 respectively.

Discussion

Comparison of haplo- and diplo-sporophytes. The relationship between the nuclear and cell sizes has been

discussed by many previous authors. In *Oryza* NAKAMURA (1933) compared several characters in his haplosporophyte and one diplosporophyte which was raised from a single seed obtained through the back cross of the haplosporophyte with the pollen grains of the mother diplosporophyte. His data about the diameter in the root tip cells do not agree with mine, but the ratio of the volume of the haploid cells to that of the diploid cells is in the main coincident with the result of the present observation.

It is very interesting that sizes of the root tip cells 1) of the original haplosporophyte, 2) of a haploid root of the chimerical culm, and 3) of the haploid tissue of a sectorial chimerical root resembled each other, and also that the thickness of the diploid and of the haploid roots from a chimerical culm resembled each other, while the root from the original haplosporophyte and the haploid part of the sectorial chimera are thinner than those of the diplosporophyte as well as the diploid part of the sectorial chimera. These data seem to show that a) the haploid root of the chimerical stock is thicker than that of the original haplosporphyte under the influence of the diploid tissue in some way and b) in the chimerical root haploid meristematic tissue in the region of the growing point may be affected by the vigorous diploid meristematic tissue, so that the haploid part cannot develop so much as in the pure haploid root in one and the same stock. In all cases the haploid cells are of similar size, and always smaller than the diploid cells.

The origin of bud-variation. There are several cases to be considered about the cause of bud-variation with chromosome duplication. According to ICHIJIMA (1931) temperature is one of the factors in the case of rice plants. In the present case no experimental treatment was made, but in the sprouting period of the original plant in 1939 the temperature was particularly high as mentioned before, so that the water temperature in the pot out-of-doors was unusually high, and it is suggested that this was the cause of the chromosome duplication in the dividing cells in the growing point of the sprout, probably due to the formation of the restitution nuclei.

The chromosome and the nucleolus. After HEITZ (1931) had pointed out the presence of the SAT-chromosome, particular attention was drawn to the nucleolar chromosomes. But if a special part of particular chromosomes only is related to the nucleolus formation in the telophase and they are concerned in the breaking of the nucleolus in the prophase of the mitosis, the nucleolar cycle in the nucleus must be independent of other chromosomes of the complement, although many authors hold the view that the nucleolus gives the material for the formation of the hyalonema or

the matrix of the chromosomes (KUWADA et alii 1934, etc.). According to the latter all chromosomes must have a relation with the nucleolus. Therefore the case of *Trillium kamtschaticum* (MATSUURA 1938) in which all chromosomes have their own nucleolus must be true for all plants. In the present case several chromosomes beside the so-called SAT-chromosome and nucleolar chromosome are found attached to the nucleolus and took a nucleolar fragment when they detach from the latter. This is in accordance with the view that all chromosomes get material for their formation from the nucleolus or the nucleoli in the prophase of the mitosis, though it is not probable that the nucleolar substance is concerned with the hyalonema formation only.

Summary

1. A fertile diplosporophyte derived from a haplosporophyte by bud-variation was separated as a clone from the mother stock. The root tip cells in the mother stock had 12 chromosomes and those in the fertile diplosporophyte had the doubled chromosome complement.

2. There appeared several culms on which the panicles had fertile grains mixed with some abortive flowers. Some adventitious roots from such culms consisted of haploid cells only, but others had completely diploid cells, and in other cases the root showed the constitution of a sectorial chimera of haploid and diploid tissue in longitudinal rows. A comparison in the sizes of the cells and the thickness of these roots was made and it was concluded that the sizes of the cells in respective tissue are firstly determined by the chromosome complements, but the propagation of the cells in the respective tissue is much influenced by other factors.

The ratio of the cell volume of the haploid cells to that of the diploid cells is ca. 1 : 1.26 which is very near to $\sqrt[3]{2}$, in other words the $2x$-cells have about 2 times the volume of $1x$-cells.

3. As the cause of the bud variation in the present case an over-heating of the water of the culture pot in the sprouting period is suggested. The $2x$-sporophytes propagate more rapidly and grow more vigorously than $1x$-sporophyte and overhelm the latter. So we can easily see that in nature haplosporophytes produced in some perennial plant, may survive until when the $2x$-bud variation appeared among them, an event which proved fatal to the $1x$-plants.

4. In early period of the mitosis, probably all the chromosomes are attached to the nucleolus either with their special points or with their end. The former are the naked or very thinly covered parts of the chromonemata, and not the surface of the hyalonema

(the chromosome matrix). The nucleolar substance is probably concerned in the various stages of the chromosome cycle for all chromosomes, and not limited to particular ones such as the nucleolar or SAT-chromosomes. The latter kinds of chromosomes have longer naked or thinly covered chromonema neck, so that they are attached more closely to the nucleolus.

Here the writer wishes to express her thanks to Prof. K. Fujii for his kind advice in the course of this investigation. Her thanks are also due to the authorities of the Kōnosu Branch of the Imp. Agric. Exper. Station for the supply of the material.

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Development of *Plasmodium praecox* in *Culex pipiens pallens*

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Introduction

A number of species of mosquitoes have previously been known to be susceptible to infection with one or another species of *Plasmodium* parasitic in birds. *Culex pipiens* is one of the well known species of mosquitoes, susceptible to such bird malaria.

Concerning the infection of this species Ishii & Suzuki (1939) have reported that oocysts were found on the wall of stomach and sprozoites in the salivary gland after the insects had fed on birds infected with *Plasmodium praecox*. However, Huff (1927–40) stressed the fact that certain individuals of *C. pipiens* possess immunity against the avian parasites *P. cathermerium* and *P. praecox* though other individuals may show very serious symptoms of infection.

Other authors also believed that individual immunity from *Plasmodium* infection exists in this species of mosquitoes.

The present work has been carried in order to investigate the cyto-histological difference in these two types of mosquitoes as regards immunity, and this paper deals with the results obtained concerning cytological changes of the malarial parasites in the stomach of *C. pipiens* after feeding and its development in both susceptible and refractory individuals.

Materials and Methods

Culex pipiens pallens Coquillett used in the present work was bred out in the laboratory over a period extending from May to June, from larvae and pupae collected from ditches in the vicinity of Tokyo.

The mosquitoes were fed on infected carnaries (Serums canarius) and after being kept for 17 days with dilute syrup as food, were allowed to oviposit. The progeny of the same strain of the mosquitoes were used, while the parasites used were a strain of *Plasmodium praecox*.

It was very necessary to feed the mosquitoes on such birds as show numerous gametocytes in their blood. So the birds showing

an average of at least one gametocyte per microscopical field were used for the feeding experiments.

The mosquitoes were about 3–6 days old when fed on the infected bird, and the engorged individuals weighting ca. 2.7 mg were separated from the others, and after 3–17 days were fixed in toto in the Carnoy Lebrum solution.

The sections were cut 12–14 micra in thickness, and stained with Heidenhain's iron-alum haematoxylin.

Observation

In order to learn the nature of the parasites, their appearance and fate in the mosquitoes after feeding were examined by means of microscopical studies.

The parasites taken into the stomach of the mosquitoes will be described in the following section.

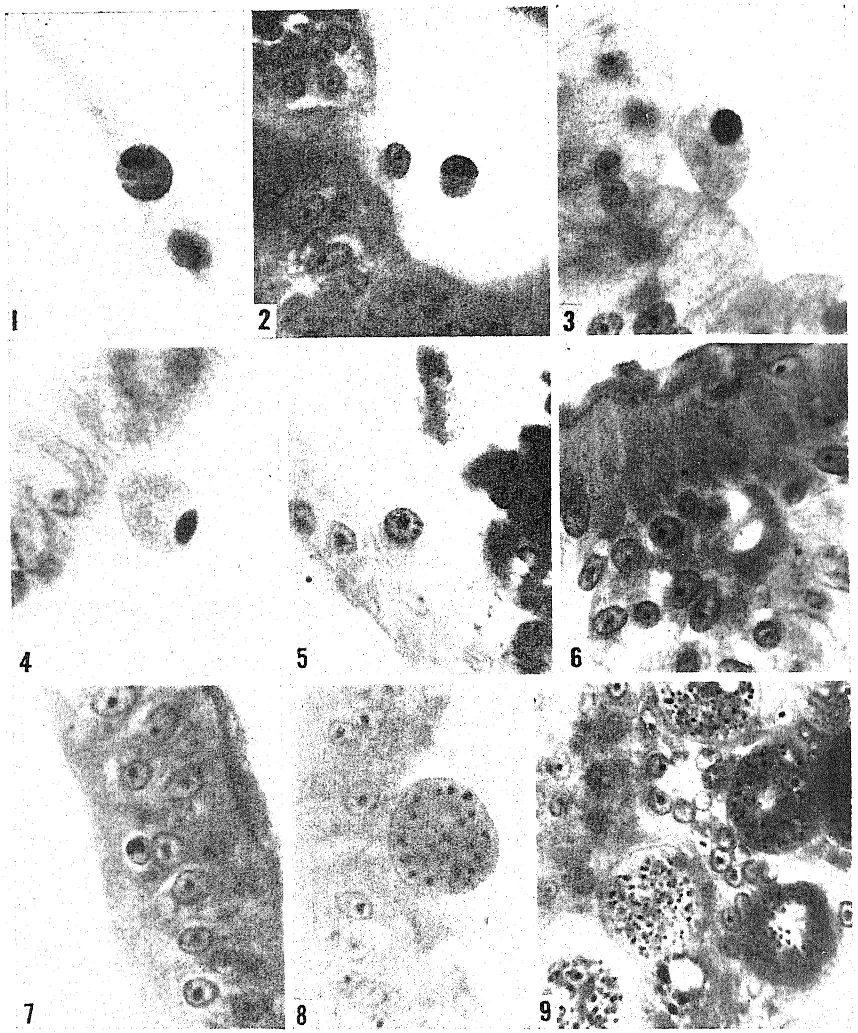
Figs. 1–2 represent zygotes which were found in a mosquito on the third day after feeding. Two similar nuclei can be seen in the zygote (Fig. 1). In the more advanced stage (Fig. 2) two nuclei are closely conjugated at one side of the zygote.

The zygote in which the conjugation of gamete nuclei is completed, (then ookinete), grows to a considerable size, the nucleus always staining more or less deeply (Figs. 3, 4). The ookinetes are situated along the inside of the stomach.

In the next stage, the ookinete appears smaller in size than in the preceding stage and assumes an elongated oval form, while its nucleus comes to show beaded chromatin threads, and has large karyosome (Figs. 5, 10). From this stage onward, the ookinetes are more or less flat in shape, and the chromatin threads in the nucleus are rather difficult to see, but one large karyosome still remains staining deeply (Fig. 11).

The ookinetes before oocyst formation are found between the cells on the stomach wall. Spherical in form and with a distinct base, they are more deeply stained than the epithelial cells. At this stage the chromatin thread disappears in the nucleus, while only one large karyosome remains (Figs. 6, 12). From this stage onward, two karyosomes are found in the nucleus of the ookinete, one being roundish and the other somewhat rod-shaped (Figs. 7, 13). As the oocysts grow, the number of karyosomes increases gradually as shown in Figs. 14, 15.

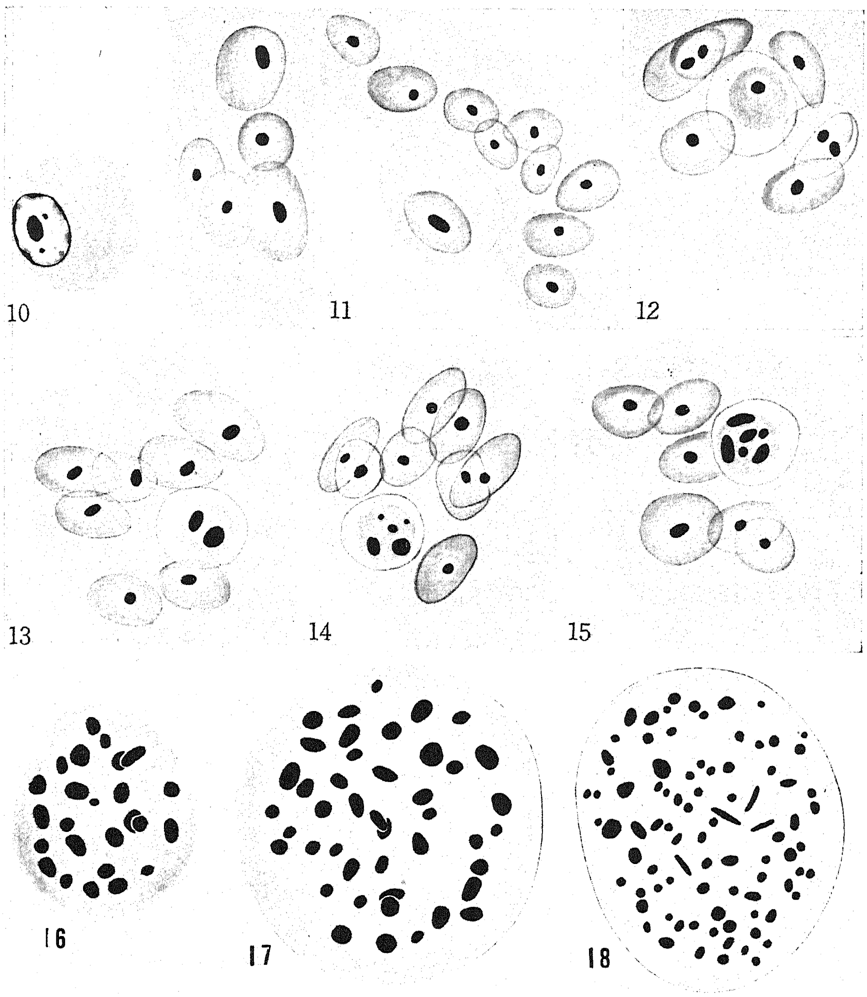
Late stages of development are shown in Figs. 8, 9, 16–18. Here the oocystes are already found outside of the stomach wall, while the karyosomes have increased in number and are scattered widely within the oocyst. Almost all the karyosomes in the younger



Figs. 1–9. 1. Zygote (ookinete) found in the stomach of a mosquito, 3 days after feeding. Two nuclei conjugating. 2. More advanced stage, fusion of the unclei proceeding. 3, 4. Young ookinetes found along the inside of the stomach. 5. Late stages of ookinetes. Nuclear frame work appearing to sight. 6. Ookinetes found intercellularly in the stomach wall. 7. Two karyosomes seen in oocysts. 8. Oocysts lying outside of the stomach wall of an insect 5 days after feeding. 9. More developed oocysts found in an individual on the 7th day after feeding. Karyosomes are smaller in Figs. 9 and 18 than those of Figs. 16 and 17.

oocysts are large in size (Figs. 8, 16, 17). Those of the older oocysts however are found in various sizes, a few of them being larger and the majority small (Figs. 9, 18).

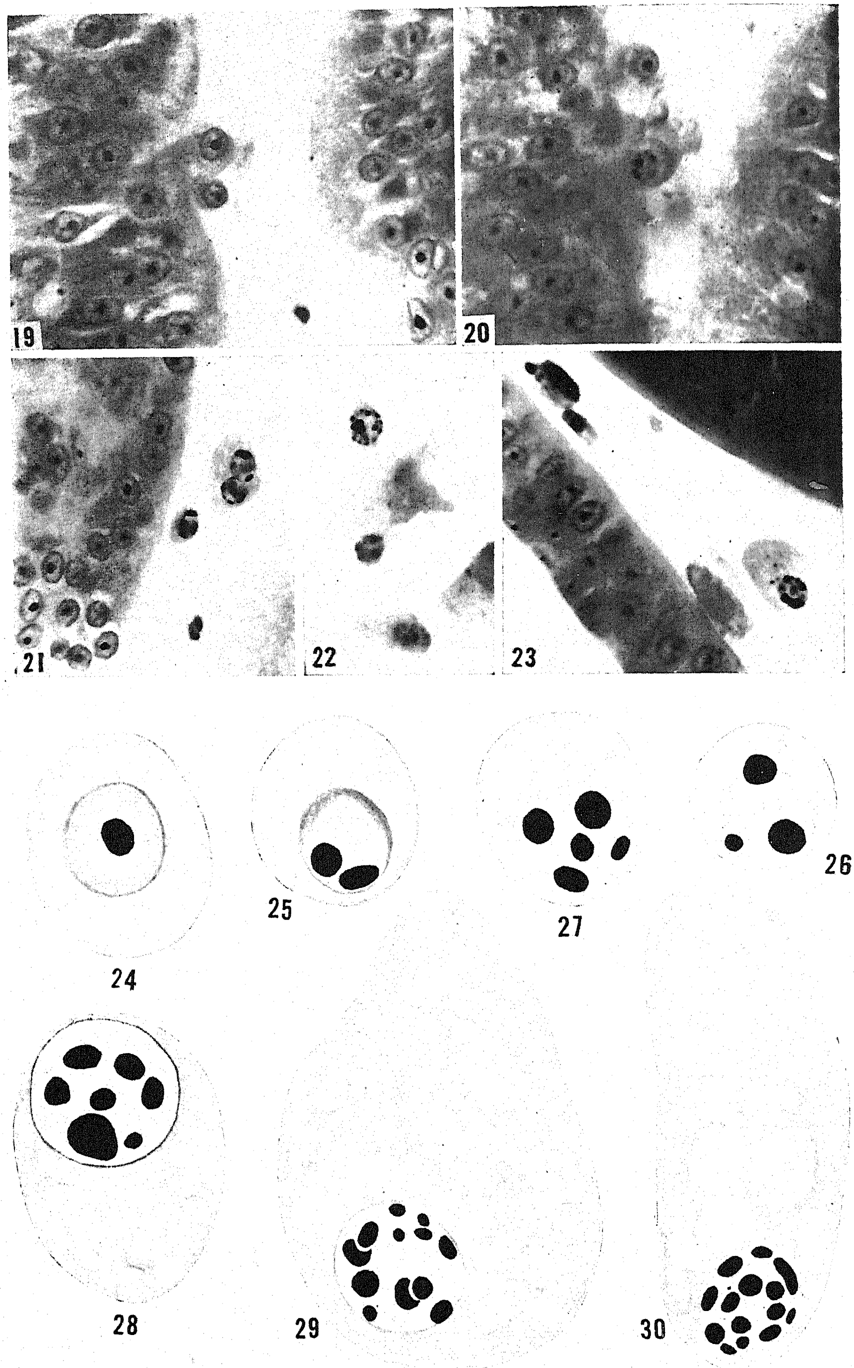
It may be interesting to note that the parasites in the stomach of the mosquitoes showed a particular development. The ookinetes



Figs. 10–18. **10.** Stage as seen in Fig. 5. **11.** Ookinete entering into the epithelial tissue. **12.** Ookinetes as in Fig. 6. **13.** Stage as in Fig. 7. **14, 15.** Younger oocysts showing increase of karyosome number. **16, 17.** Stages as seen in Fig. 8. **18.** Stages as seen in Fig. 9.

remained along the inside of the stomach wall, as seen in Figs. 19, 24. They are of elongated oval form, with a nucleus in which no trace of the chromatin threads is seen, only one large deeply stained karyosomes being found.

Judging from the size, form and staining reaction of the nucleus, the ookinetes found within the stomach correspond to those seen among the epithelial cells of the stomach as mentioned above. But the writer assumes that these ookinetes developed in the stomach but failed to penetrate the stomach wall.



Figs. 19–30. 19, 24. Showing development of ookinetes and oocysts both having a large karyosome and no chromatin threads. 20, 25. Oocysts having two karyosomes. 21, 26–28. Young oocysts showing increased number of karyosomes. 22, 23, 29, 30. More advanced stages of oocysts, the cytoplasm showing a number of vaculoles as a sign of degeneration.

Figs. 20, 21, 25–27 show the early stage of the oocysts in the individuals that passed four days after taking the infecting meal. These oocysts in which the karyosomes increased in number correspond to the young oocysts found between the cells of the stomach wall.

More advanced stages of oocysts found in the stomach are given in Figs. 23, 28–30. Here a number of koryosomes were scattered widely in the nucleus and some vacuoles are seen in the cytoplasm.

Looking over the results of the present examinations on *P. praecox* in the stomach of *C. pipiens pallens* after feeding, it may be noted that the young oocysts were found within the stomach of the mosquitoes.

The writer is of the belief that such young oocysts within the stomach of the mosquitoes degenerate and can not be developed further.

As stated, a careful examination of the parasites was made in the mosquitoes from 3 days to 17 days after feeding. In all 163 mosquitoes were sectioned and observed by the method described above. 101 individuals were found to have oocysts of the parasites, while the rest (62) had no oocysts.

Though the development of the parasites is generally effected by a common process as stated above, it seems that the process differs with different *Culex* individuals with the time elapsed after feeding. From this viewpoint the development of the parasites was observed in the stomach of each mosquito individual either at the same time or at a different time after feeding.

The mosquitoes were examined daily from the fourth day after feeding onwards and the presence or absence of the ookinetes was observed (Table 1).

Table 1

Days after feeding	No. of insects having ookinetes in the stomach	No. of insects having no ookinetes in the stomach
4	31	9
5	20	6
6	20	3
7	11	3
8	10	2
9	7	3
10	9	2
11	10	1
12	3	—
13	7	1
14	1	—
16	2	1
17	1	—
Total	132	31

As seen in Table 1 the ookinetes of the parasite remained in the stomach until the 17th day.

The results may be further summarized according to the nature of the ookinetes in the stomach. The difference between normal and degenerate ookinetes was usually quite easily observed.

Degenerating ookinetes can be distinguished either by their irregular outline, dissolution of the nucleus or appearance of vacuoles in the cytoplasm. Normal ookinetes have a smooth, regular boundary and a distinct nucleus. The ookinetes in the stomach of individual mosquitoes can be divided into three types as seen in Table 2, where only one is of type I, while 14 are of type II and 117 of type III.

Table 2. Numbers of mosquitoes belonging respectively to the 3 types 4–17 days after feeding

Days after feeding	Type I	Type II	Type III
4	—	11	20
5	—	2	18
6	1	—	19
7	—	—	11
8	—	—	10
9	—	—	7
10	—	—	9
11	—	1	9
12	—	—	3
13	—	—	7
14	—	—	1
16	—	—	2
17	—	—	1
Total	1	14	117

Type I = normal ookinetes found.
Type II = both normal and degenerating ookinetes found.
Type III = only degenerating ookinetes found.

Though the ookinetes remained in the stomach until the end of the 17th day, almost all of them were observed in a state of degeneration.

Next, the further development of parasites in the same mosquitoes was examined according to the existence of oocysts. The following four types were obtained on the basis of development of the parasites.

Type A, development of the parasites is incomplete, but early stages of the oocyst were observed in the stomach, as seen in Figs. 21–23, 25–30.

Type B, besides the production of oocysts in the stomach as in case of type A, oocysts were found outside of the stomach wall.

Type C, production of the oocysts was observed outside of the stomach wall only.

Type D, oocyst production was not observed, no oocysts being found at all either in the stomach or outside the stomach wall.

The results of the observation of the individuals having no ookinetes in the stomach will be summarized in Table 3, according to the four types above mentioned.

Almost all of the mosquitoes had oocysts, there being only 3 individuals which had no oocysts in the outside of the stomach wall, namely, one of type A and 2 of type D. The occurrence of 2 individuals of type D may be ascribed to experimental error.

The results of the observation of the individuals which had ookinetes in the stomach are given in Table 4.

As seen in Table 4, we obtained 45 individuals of type A, 51 of type B, 22 of type C and 14 of type D. An interesting fact is

that about half the total number of the mosquitoes failed to show development of oocysts.

Table 3

Days after feeding	Type A	Type B	Type C	Type D
4	—	4	5	—
5	—	5	—	1
6	—	1	2	—
7	—	—	3	—
8	—	2	—	—
9	—	3	—	—
10	—	1	—	1
11	1	—	—	—
13	—	—	1	—
16	—	1	—	—
Total	1	17	11	2

Table 4

Days after feeding	Type A	Type B	Type C	Type D
4	13	11	4	3
5	7	8	4	1
6	4	8	5	3
7	5	4	1	1
8	—	7	2	1
9	2	3	1	1
10	5	2	—	2
11	4	1	4	1
12	1	2	—	—
13	3	2	1	1
14	—	1	—	—
15	1	1	—	—
17	—	1	—	—
Total	45	51	22	14

Thus in spite of the fact that all the mosquitoes were fed on the same infected bird, the degree of development of the parasites in the insects varied in such a way that division into these four types was necessary.

Discussion

Studies on the infection of mosquitoes with bird malaria have been reported by several investigators, and various species of mosquitoes have been known to be susceptible to infection with one or other species of *Plasmodium* in birds. Above all, *C. pipiens* known as an insect susceptible to infection with bird malaria, has been studied to solve the problems arising in this connection.

Here, the results of the work of other authors will be compared with our own findings in the case of *C. pipiens pallens.*

As stated in the previous pages, a careful examination of the parasites in the mosquitoes was made from 3 days to 17 days after feeding. According to their fate and appearance in the stomach after feeding seem to be divided into two types, as seen in Table 1. On the one hand there were some ookinetes remaining within the stomach of the mosquitoes and on the other hand there were none.

In his observation on the morphological changes in the various elements of the blood meal in the stomach of *C. pipians* by means of the cytological examination of individuals both susceptible and refractory to infection with *Plasmodium cathermerium* and *P. relictum,* Huff (1934) reported that normal and degenerating

ookinetes were found in both susceptible and refractory individuals for about the first 30 hours, that the number of normal ookinetes in both kinds of mosquitoes decreased with the passage of time and that *P. relictum* remained for a longer time than *P. cathermerium.*

However, in our examination the following marked features of the ookinetes in the stomach of mosquitoes were observed.

Normal ookinetes were present up to the 11th day after feeding, but not in such large numbers as in the period shortly after feeding, and degenerating ookinetes were also present up to the 17th day. The development of the parasites in the mosquitoes is not the same in all individuals after feeding, and we may distinguish by means of microscopical examination four types of development of *Plasmodium praecox* in *C. pipiens pallens*, as seen in Tables 3 and 4.

Formation of the oocysts did not occur in the stomach in both A and D types, but the formation of oocysts up to the early oocyst stage in the stomach of mosquitoes was observed in type A.

The individuals of type B had oocysts in the outside of the stomach wall besides young oocysts in the stomach.

In the individuals of type C, the parasites were able to undergo normal sporogamy and the oocysts were found outside the stomach wall. And then, in the individuals of type D, it was shown that the parasites can only develop as far as the ookinete stage in the mosquitoes.

The individuals of both types A and B which showed the presence of young oocysts in the stomach were found to be 114 out of the total number of mosquitoes (cf. Tables 3 & 4).

From the foregoing facts, it may be believed that *P. praecox* can be developed as far as the early stages of the oocyst in the stomach of *C. pipiens pallens*. From this it may be deduced that the activity of *P. praecox* in *C. pipiens pallens* is a matter of degree, as above mentioned.

The intracellular penetration in the stomach wall by the ookinetes has been reported by several authors, but the writer has not yet seen any ookinetes within the epithelial cells, but has observed the ookinetes to be present between the cells of the stomach wall.

The process of intercellular penetration by the ookinetes was also observed by the present writer.

The number of oocysts developing in the stomach of the mosquitoes after feeding has been investigated in detail by several authors.

The experiments of Huff indicated that the number of oocysts developing in the stomach of *C. pipiens* was determined not entirely

by the number of gametocytes ingested, but to some extent by the physiological make-up of individual mosquitoes.

Shah et alii (1934) stated, however, that the percentage of infected mosquitoes increases directly with the number of gametocytes ingested and that with increase of number of gametocytes ingested the immunity of the mosquitoes was broken down and the number of refactory mosquitoes decreased.

In their comparative studies on the susceptibility of autogenous and anautogenous races of *C. pipens* with *P. relictum* Roubaud and Megger (1933, 34) reported that the former were observed to become infected in all cases, but the latter in only about 40% of the cases investigated. Tate and Vincent (1932, 34) reported that autogenous and anautogenous races of *C. pipiens* behaved similarly as the strains of *P. relictum,* but the strains of malaria differed in their infection rate to the mosquitoes. Lumasden and Bertran (1940) stated, as a result of experiment, that the highest average oocyst count tends to preceed the highest gametocyte count per unit volume. Therefore, the number of oocysts developing in the mosquitoes must presumably be influenced by variations of gametocyte count in a given volume of blood. As noted above, the number of oocysts developing in the stomach of the mosquitoes has been discussed in various ways by other authors. Considering the results obtained, the present writer is of the opinion that variation of development of the parasites in *C. pipiens pallens,* as Huff concluded in other materials, may be due to the grade of resistance of the mosquitoes to infection with *P. praecox.*

Summary

1. The development of *Plasmodium praecox* in *Culex pipiens pallens* from 3 days to 17 days after feeding on the blood of infected canaries was studied cyto-histologically.

2. Normal ookinetes remained in the stomach of *C. pipiens pallens* until 11 days after feeding, but degenerating ookinetes were observed on the 17th day after feeding.

3. The formation of young oocysts of the parasites was observed within the stomach, but further development could not take place.

4. The development of *P. praecox* in *C. pipiens pallens* is a matter of degree, namely, four types of development were observed in the individual mosquito of this species.

5. It was suggested that *C. pipiens pallens* has two races or two groups of individuals of the same race with regard to immunity from infection with *P. praecox.*

In conclusion, the writer wishes to thank Prof. H. Kikuchi of the Japanese Army Medical College, Tokyo, for his kindness and help in facilitating the present work.

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Studies on the Plasmolysis Form

By

Noboru Takamine

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Introduction

In the plasmolysis, the separation of the cytoplasm from the cell membrane generally does not take place smoothly, but with difficulty, overcoming the resistance due to adhesion, so that there appear various plasmolysis forms and destructive figures of the cytoplasm. As such figures there may be mentioned Hecht's fibers[1], arabesque pattern on the cell wall (here shortly wall pattern), various types of plasmolysis including tonoplast plasmolysis and

1) Chodat et Boubier (1898), Hecht (1912), Hansteen-Cranner (1922), Weis (1925).

cap plasmolysis[1], protuberance or myelin figures[2] etc. Some of these phenomena were investigated by the present writer and the results obtained were reported in the previous papers (1940 a, b).

In the results of investigations of these plasmolytic phenomena and in their explanations, there are often found certain disagreements, especially when the investigations are concerned with plasmolysis form. This discrepancy is, however, rather to be attributed to the difference in the characteristics and age of material, its treatment, the procedure of preparation, the kind and concentration of plasmolyticum and the conditions under which the microscopical observations were made. Derry (1930) and more emphatically Borriss (1938) have indicated that great care must be taken on these points, especially when the plasmolysis form is concerned. Kamiya (1939) has observed in *Allium cepa* that the plasmolysis form differs in various parts of the inner epidermis, and attributed this difference to the inner condition. These causal relations between the plasmolysis form and its governing factors were investigated in *Allium cepa* by the writer (1940a) and it was pointed out, how very careful one should be, when the studies on the plasmolysis form are carried out. Based on the experimental observations we tried there to explain the etiology of the various types of normal and abnormal plasmolysis by assuming a competition between the cohesion of the cytoplasm itself and its adhesion to the cell membrane, and summarized them with Schemata 1–8[3]. According to this interpretation the various types of plasmolysis can not be explained merely by the viscosity or adhesion of the cytoplasm each alone, but rather by the combination of these forces.

In the present investigation which is the continuation of the previous one the processes of the normal and abnormal plasmolyses, especially those of the tonoplast plasmolysis and cap plasmolysis are more precisely studied than in the previous investigation with special reference to the characteristics of the material and to the conditions under which the experiments are carried out.

Though the questions sometimes remain not precisely determined, whether or not the physical nature of the cytoplasm is inferable from the plasmolysis form or plasmolysis time[4], it seems not improbable that by this method some difference can be seen in certain characteristics of the related species or varieties or between plants or tissues young and old, if the experiments are carried out

1) Höfler (1928, 1934, 1939). Weber (1930).
2) Küster (1927).
3) An additional schema is given in the Part IV in the present paper.
4) Borriss (1938).

with every precaution, and if the results obtained are considered very carefully. In the remaining part of the present paper the following problems on this line are treated, applying the method of the plasmolysis form:

a) The difference between the upland and paddy rice plants, which are ordinarily cultivated under different ecological conditions, especially as concerned with water relation in the soil.

b) The physical nature of the cytoplasm of a certain variety of *Linum usitatissimum,* which is resistant to the wilt disease, in comparison with corresponding characters of some susceptible varieties.

c) Some differences in the physical nature of the cytoplasm between some species of *Triticum,* in connection with the resistance to fungus disease similar to that in the case of the flax.

Part I

Tonoplast plasmolysis and cap plasmolysis in the inner epidermis cells of *Allium cepa*

The preliminary experiments show the following facts:

Generally the normal plasmolysis begins with the concave form, and though some differences in the plasmolysis time are perceivable, it turns to the convex form sooner or later. Such process of the plasmolysis is common, when saccharose or $CaCl_2$ solution is used as the plasmolyticum. Though in some cases some disturbances occur at the beginning, the normal process is soon recovered. In the entirely healthy material, the cytoplasmic movement is seen even 24 hours after the material was put in the plasmolyticum. When, however, salts of alkali metals such as KCl are used as plasmolyticum, the plasmolyzed cytoplasm more or less swells up, because it has intrability[1] to the salts to a certain degree, and the osmotic water absorption occurs. Even in the case when the plasmolysis begins in the Schemata (1) and (2)[2], it does not last long. Therefore, the abnormal types of plasmolysis appear sooner or later, among which may be mentioned especially the tonoplast plasmolysis and cap plasmolysis here.

Material

As reported in the previous paper, a close relation exists between the plasmolysis form and the condition of the experiment or the characteristic of the material.

1) According to the nomenclature of Höfler.
2) Takamine (1940a).

As in the present investigation the inner epidermis of onion bulbs was used, which had been stored in a cellar for a certain period before use, it may not be impossible to assume that the experimental results we obtained depended in some measure upon the condition under which the preservation had been made[1].

For the sake of convenience of description the materials are classified into 3 groups according to the plasmolytic nature of the cytoplasm.

A. Material taken directly from field. In this paper, this material is sometimes called "fresh material". In a 1 mol saccharose solution the concave form of plasmolysis appeared at first, and then it turned smoothly to the convex form. In a 0,5 mol KCl solution the concave form appeared and lasted pretty long, and a remarkable figure of Hecht's fibres was observed. Such a material is to be regarded as healthy one.

B. Bulbs harvested in the previous autumn, stored in a cellar for a certain period and subsequently preserved in saw dust (17°–22°C) for one month before use. The plasmolysis occurs with some difficulty in a 1 mol saccharose solution. In a 0,5 mol KCl solution the plasmolysis begins in the concave form, and after a long duration it turns to the convex form. It often becomes the tonoplast plasmolysis soon after the beginning. Such a material is regarded to be quasi-healthy.

C. Unhealthy material which is found frequently in the following cases. 1) Bulbs which have been stored without any special care since the time of harvest in the previous autumn. 2) The material of *A* or *B* class which has been stored at a high temperature or in a dry condition. In these cases the plasmolysis begins with much difficulty in a 1 mol saccharose solution and the concave plasmolysis or sometimes tonoplast plasmolysis is visible. When the material is immersed in a 0,5 mol KCl solution, it shows the tonoplast plasmolysis or convex plasmolysis[2] sooner or later.

In the experiments the inner epidermis of the second leaf was always used, and the section was made with the same method as reported in the previous paper (p. 303).

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1) Krassinsky (1930) and Höfler (1934, p. 96) have noticed that the epidermis plasm of the *Allium cepa* shows some seasonal changes, and the cap plasmolysis is not always observed even in the case of the same kind of material being used. Döring (1932, p. 425) has remarked that when *Allium cepa* is stored in a cool dark place, cap plasmolysis does not occur, while it appears in the material stored in a warm place.

2) The cytoplasm in this convex plasmolysis is, however, not quite healthy in the strict sense.

Glass-redistilled water was used to prepare the plasmolyticum, and the chemicals of the same source and the slides and cover glasses and other glass wares of the same quality, as those reported in the previous paper, were used.

1. Tonoplast plasmolysis

The tonoplast plasmolysis occurs in material *C*, especially easily in a KCl plasmolytic solution. It seems likely that this plasmolysis has some relation to the cap plasmolysis, as described below.

In regard to the occurrence of the tonoplast plasmolysis three cases may be distinguished (Fig. 1 a, b. Obtained from material of *C* group).

a) The contraction of tonoplast precedes the separation of the cytoplasm from the cell membrane. This phenomenon is somewhat similar to the vacuole contraction, and the appearance shows that this type belongs to Schema 4 in the previous paper (1940a). This type may thus be added to this schema as a new type. In the meanwhile, the appearance remains like Schema 7, if the separation of the cytoplasm was tardy, but after the separation of cytoplasm it changes to the cap plasmolysis.

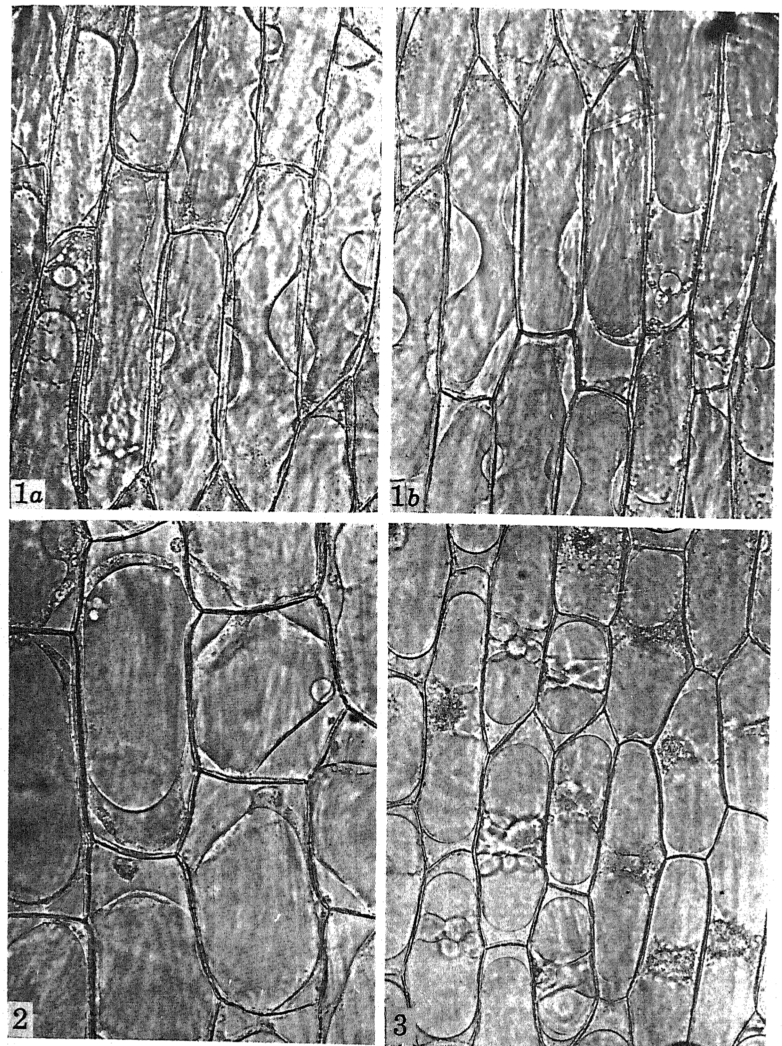
b) At the beginning of the plasmolysis, the cytoplasm separates from the cell membrane, and this is then suddenly followed by the tonoplast contraction. In this case too, the appearance is like one belonging to Schema 4. Afterwards it will possibly change to the cap plasmolysis.

c) When the tonoplast contraction occurs very quickly, the states such as shown in Schemata 6 and 8 result.

In every case of tonoplast plasmolysis, the cytoplasmic body between the surface of the cytoplasm and the tonoplast is outstretched, and in the cases of *a* and *b* fine microsomes and small droplets of the cytoplasm are found in this outstretched part. In the case of *c*, besides the fine microsomes there appear many pretty large drops and sometimes thick fibres, which resemble Hecht's fibres (Schema 8).

Though the tonoplast plasmolysis may possibly be caused by the increased adhesion due to the dry condition, it seems also likely that in most cases it is due to a high intrability, the semipermeability of the tonoplast being not wholly lost. The three cases (*a*, *b* and *c*) of tonoplast plasmolysis mentioned above occur, therefore, mainly according to the degree of intrability. When the intrability attains to the maximum, the tonoplast plasmolysis appears as that shown in Schema 8 or as the vacuole contraction shown in Schema 7.

When the material showing the tonoplast plasmolysis *a* or *b* in a 0,5 mol KCl solution, a material in which the cytoplasm has certainly no intrability, is transferred into a 0,5 mol $CaCl_2$ solution, the cytoplasm begins to contract and turns to the convex forms.



Figs. 1–3. 1. Material *C*. Tonoplast plasmolysis. 2. Material *B*. Tonoplast plasmolysis. The pH-value of the KCl solution was adjusted to 7,3 with KOH. 3. Material *A*. Tonoplast plasmolysis. The pH-value of the KCl solution was adjusted to 7,3 with $KHCO_3$.

Even in a saccharose solution such a contration of the cytoplasm is visible in some degree. This phenomenon may be interpreted as follows.

The whole enlarged cytoplasmic body is regarded as an osmotic system. Its outer surface is permeable to KCl, while the tonoplast is impermeable to it. As the former is, however, impermeable to both $CaCl_2$ and saccharose, water is osmotically taken out from the cytoplasm, when those solutions are used as the second plasmolyticum. The action of $CaCl_2$ solution in the case of the cap plasmolysis can be explained in a similar manner. The opinion of Höfler (1939) on this phenomenon will be considered in the following section on the cap plasmolysis.

The intrability of the cytoplasm to KCl may be regarded as that to $K^{\cdot}$ or the ion pair $K^{\cdot}$ and OH' of the basic part, hence the tonoplast plasmolysis is easily caused even in the case of the healthy material of *A* or *B* class, if the pH-value of the KCl solution is adjusted to 7,3 with $KHCO_3$ (Figs. 2, 3). When the pH value is increased by a further addition of KOH, the cytoplasm in the state of tonoplast plasmolysis, swells up and partially dissolves.

2. Cap plasmolysis

As pointed out by Höfler (1928) the cap plasmolysis is caused by salts of alkali metals, such as K-salt, but not by a Ca-salt. In the present experiments the cap plasmolysis occurred frequently in

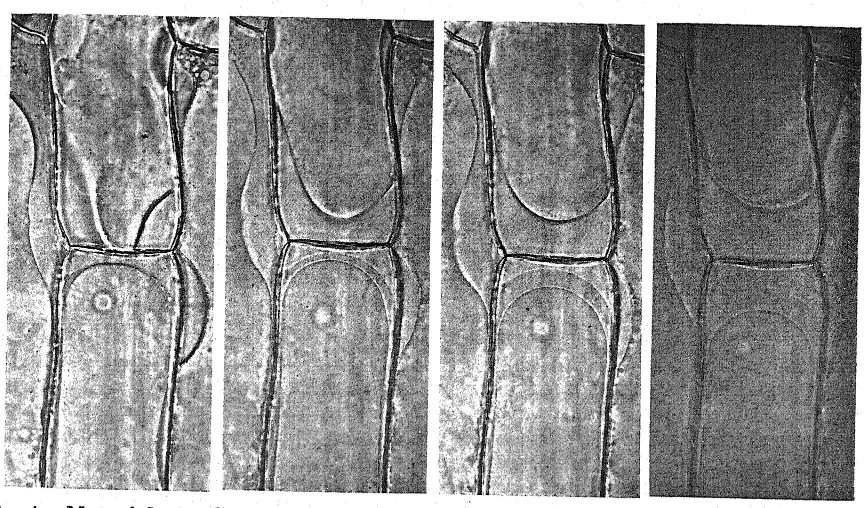


Fig. 4. Material *A*. Successive of stages the swelling of the cytoplasm at the narrowed region of the plasmolyzed cell. The cap formation occurs in Schemata $1 \rightarrow 2 \rightarrow 3$.

the material of *B* class, but more easily in that of *C* class. Even in the material of *A* class, however, it often appeared with the lapse of time after the immersion of material in the plasmolyticum. The etiology of the cap plasmolysis may be classified as follows.

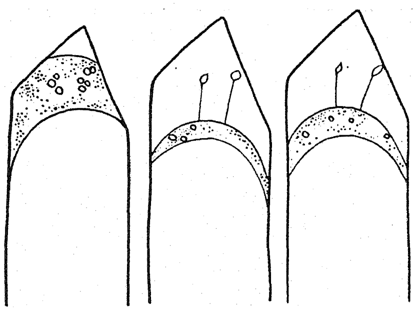
a) The course shown in Schemata 1→2→3. When cells in the material of *A* class are plasmolyzed in a KCl solution, the plasmolysis begins with the concave form and then turns to the convex form. The plasmolyzed cytoplasm first appears like a thin layer and then gradually swells up at both narrowed regions of the cell (Fig. 4).

Two cases are distinguishable in regard to the direction of the swelling up of the cytoplasm. In one case the swelling proceeds toward the outside, and in the other case towards the inner side, causing the vacuole contraction. In both cases comparatively small microsomes are found scattered in the wide stretched cytoplasm, though sometimes that part of the cytoplasm appears pretty clear.

The cap plasmolysis of this type *a* takes a pretty long time for its complete formation.

According to Höfler (1928, 1934) the swelling of the cytoplasm is necessary as a first process to cause a cap plasmolysis. His cap plasmolysis probably belongs, therefore, to our case *a*.

b) The course shown in Schemata 1→4→5 (Figs. 5, 6). This kind of cap plasmolysis often occurs in *C* class material. In this case the concave plasmolysis shown in Schema 1 sometimes lasts long, and sometimes not. In either case it changes sooner or later



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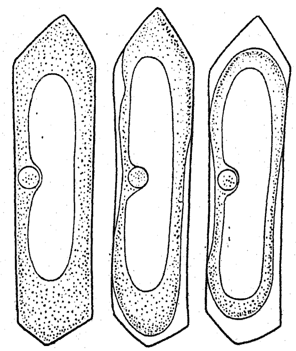

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Figs. 5–6. 5. Material *C*. The cap formation occurs in Schemata 1→4→5. The plasmolysis begins in the concave form which is followed by the toneplast plasmolysis and by the perfect separation of the cytoplasm from the cell membrane. Camera lucida drawing. 6. Material *C*. Tonoplast plasmolysis followed by the separation of the cytoplasm from the cell membrane. The cytoplasm retains its increased thickness not only at the narrowed regions, but also along its long sides. Camera lucida drawing.

to tonoplast plasmolysis of *a* or *b*. Many microsomes and *A* drops[1] come togather in the stretched cavity of the cytoplasm, often produced by the contraction of the tonoplast. Then follows the complete separation of the outer surface of the cytoplasm from the cell membrane; the cytoplasm becomes lost the concave form and rounded

1) The explanation of this kind of drops will be made below.

off, retaining its increased thickness. Such a change occurs rather frequently at both narrowed regions of the cell, but sometimes also along its long sides. When the cell is not long in shape, but rather nearly square, the thickened cytoplasm rounds off spherically. Sometimes the plasmolysis results in cap plasmolysis at last. In this case the plasmolysis begins with the tonoplast plasmolysis which is followed by the plasmolysis form shown in Schema 7, and then the cytoplasm becomes contracted, being separated from the cell membrane.

c) This type of cap plasmolysis frequently occurs in a material of class *B* showing some approach to *C* in the characters of classification. In the *C* material, which is apt to show tonoplast plasmolysis, this type of plasmolysis is only rarely met with. In the convex plasmolysis (Schema 2) where Hecht's fibres are observed, it is frequently seen that the cytoplasm is projected in various forms towards the vacuole from its main body (Küster, 1927) (Figs. 7, 8). These projections have been called protuberances or myelin figures. In the present experiments three forms of such cytoplasmic projections were observed[1]. They are (1) ligulate, (2) spherical and (3) filiform. All these projections vibrate actively, and the forms are interchangeable. They appear mainly at the narrowed regions of the cell, but sometimes also along the long sides[2] (Fig. 7).

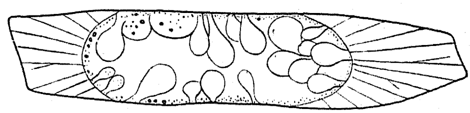


Fig. 7. Material *B*. Ligulate and spherical protuberances. Hecht's fibres. Camera lucida drawing.

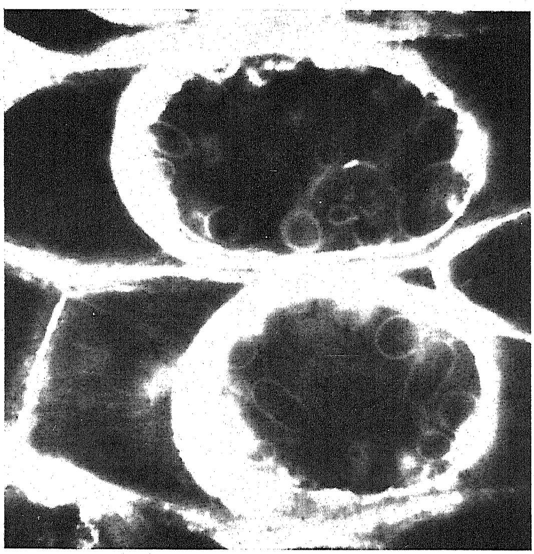
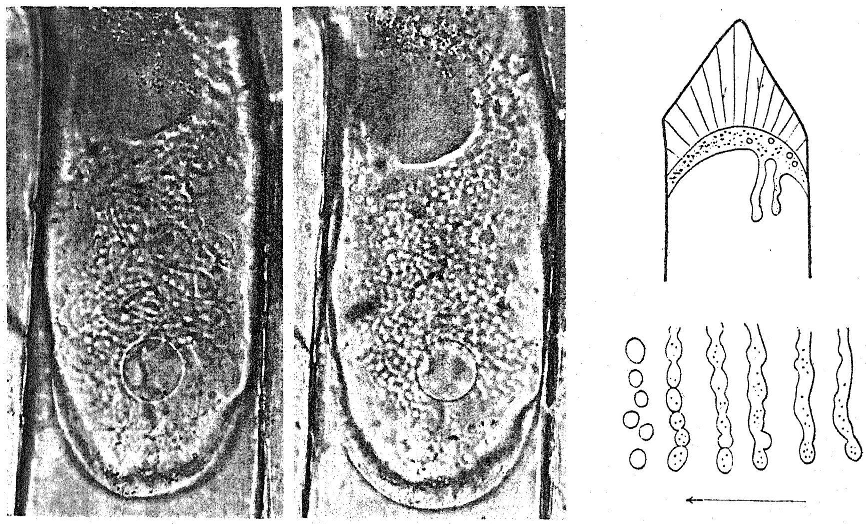


Fig. 8. Material *B*. Spherical protuberances. Hecht's fibres. Photographed with dark field illumination.

1) Yasui (1938) has found that lipoid drops, which come out from the tapetal and pollen mother cell nuclei and chromosomes and stained with acetocarmine, show various myelin figures very similar to those observed in the present investigation.

2) Gicklhorn (1932, p. 32).

The filiform projections are of various diameters and present a snake-like motion. They become easily constricted at numerous points in a beaded form, and then are segmented into many pieces of small spherical bodies and scattered in the vacuole (Figs. 9, 10).



a 9 *b* 10

Figs. 9–10. 9. Material *B*. Filiform protuberances in the vacuole (*a*). They are segmented into many pieces of spherical bodies (*b*). **10.** Material *B*. Successive stages of the constriction and segmentation of a filiform protuberance at one narrowed region of the cell. Somewhat schematised drawing.

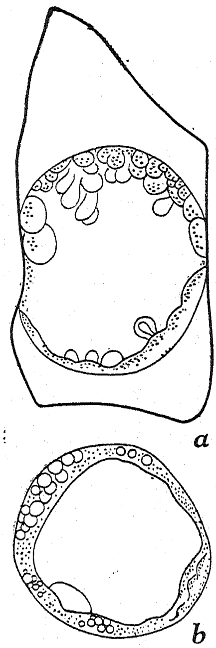
The droplets in the vacuole shown in Fig. 4 of Höfler (1939) seem to be the bodies of the same nature. In our cell which is indicated in Fig. 9, the segmentation occurs within 10 minutes.

In the case when a NaCl or NH_4Cl solution is used as plasmolyticum, such a protuberance appears just in the same way as in the case of the KCl solution, and a tendency toward an easy appearance of the fine filiform protuberances is also recognizable, especially when an NH_4Cl solution is used.

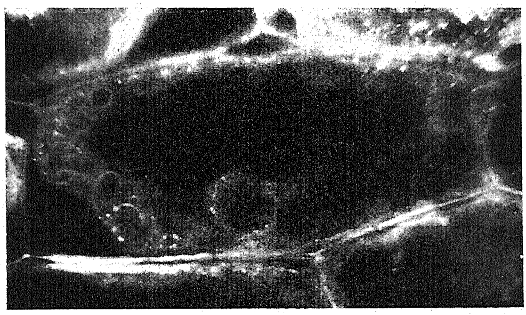
The protuberances appear more rapidly when a few drops of an NH_4OH or NH_4HCO_3 solution are added to the NH_4Cl solution to make its pH-value be 7,0. In this case, however, not only the filiform protuberances, but also the ligulate or spherical protuberances appear. Gicklhorn (1932, p. 23) has remarked that the ligulate and lobe protuberances appear easily when the plasmolysis occurs quickly. This result of Gicklhorn seems to have some connection with the concentration of the ion pair, $K^{\cdot}$ and OH', which may be regard to have an easy penetrability into the cytoplasm. When NH_4OH was added slightly in excess to the plasmolyticum NH_4Cl

solution, dissolution of the cytoplasm occurs and the protuberances rarely appear. It is, therefore, necessary to use NH_4OH in moderate quantity, in order to make the occurrence of the protuberance easy.

When the slides and cover glasses of the quality of soft glass are used, the protuberances appear more easily than in the case where hard glass wares are used. When material is immersed in a m/200 NH_4OH solution, the plasmolysis naturally does not occur, because the concentration of the plasmolyticum is too low, but the vacuole contraction accompanied by the occurrence of some spherical protuberances is observed. From this fact, a liquefying action of ammonia on the cytoplasm can be inferred to take place.



a b 11



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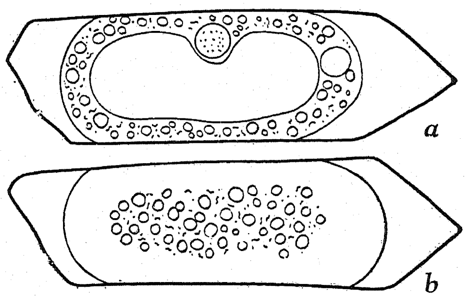


a b 13

Figs. 11–13. Material *B*. 11–12. Fusion of the spherical protuberances, forming swelled cytoplasm. 11, *a*, after 4 hours; *b*, after 5 hours. 12. Photograph of the material such as shown in Fig. 13, in dark field illumination. 13. Fusion of the spherical protuberances in the cytoplasm (*a*). Optical section of the same cell through the periphery of the upper part of the cytoplasm (*b*). Somewhat schematised drawing.

The easy occurrence of the protuberances is recognizable also when a moderate quantity of KOH or NaOH ($KHCO_3$ or $NaHCO_3$) is added to a KCl or NaCl solution, adjusting the pH-value to be 7,3. The substance of the protuberances is of somewhat high density

and shows no double refraction. It seems that it represents the fundamental substance of the cytoplasm.

According to Gicklhorn (1932), the protuberances are of the lipoid nature, which he considers come out of the cytoplasm as the result of its demixing (Entmischung). Sometimes the spherical protuberances are found assembling at the narrowed regions of the cell, forming caps. Each drop swells up and the individual drops fuse together finally with each other. The contour of the fused mass is uneven in the first stage of fusion, but it becomes smooth later, resulting in a cap plasmolysis. Sometimes the fused mass makes no cap, but swells up, to occupy the whole body of cytoplasm (Figs. 11, 12, 13).

Besides such types of protuberances, those of less density are sometimes found, projecting from the cytoplasm into the vacuole. Some of the protuberances of this kind are also observed vigorously vibrating and continually changing their form. Such protuberances are produced by swelling from many vesicles which arise in the cytoplasm.

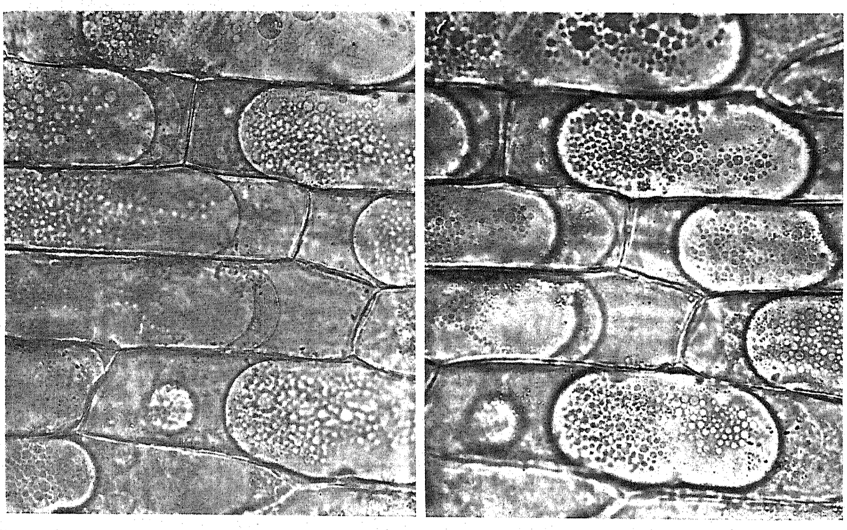


Fig. 14. Material *B*. Two different optical sections of the same material. Spherical bodies, produced from filiform protuberances by segmentation, are scattered in the vacuole. Spherical bodies are not found at the narrowed end of the cytoplasm with cap.

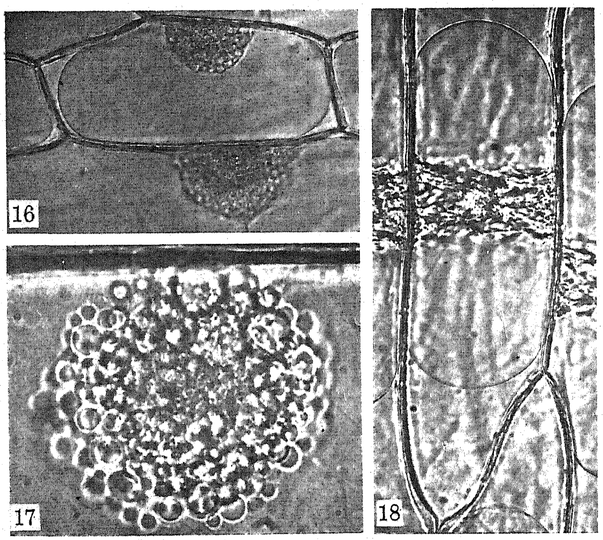
For the sake of convenience the dense protuberance described above is denoted "drop *A*" and the less dense protuberance "drop *B*". The most common protuberance belongs, therefore, to drop *A* according to this denotation.

When the spherical bodies, produced from the filiform protuberances by segmentation, are scattered in the vacuole, the cap

form of plasmolysis is apt generally not to appear. If the cap formation is limited to the one narrowed part of the cell, these spherical bodies are found distributed only in the region of the opposite end (Fig. 14). From this fact it seems that the drop *A* protuberance has a close connection with the cap formation.

Fig. 15. Material *B*. Protuberances fuse together into large masses in the vacuole. The cap is not formed.

Sometimes the drops *A* are observed assembling in a certain region of the vacuole, or fused together into large masses which are distributed throughout the vacuole (Fig. 15). In this case the cap is also not formed, if the various parts of the cytoplasm remove into the vacuole and come together in such a way, as systrophe can easily occur. In those plasmolyzed cells, the peripheral layer of the cytoplasm, is very thin and looks just like the tonoplast. It may perhaps be regarded that it is the outermost layer of the cytoplasm. If this layer retains its semipermeability to the plasmolyticum to some degree, deplasmolysis occurs and the layer becomes finally expanded to be in contact with the cell membrane.



Figs. 16-18. Material *B*. Systrophe. 17. The nucleus forms the central figure of the aggregate in the systrophe.

The protplasm which takes part in the systrophe sometimes arises from the drop *A* and sometimes from the main body of the

cytoplasm, and in most cases the nucleus forms the central figure of the aggregate (Figs. 16–18).

It is not infrequently met with that the cap plasmolysis occurs in the way not of *a*, but of *b* or *c*, when the material is somewhat unhealthy in the sense of plasmolysis.

Höfler (1934, p. 76) has remarked that in some cases the cap plasmolysis does not take place in *Allium* according to the material, and Döring (1932) has pointed out that when an onion bulb stored in a cool place is used, the cap plasmolysis hardly occurs, while it does easily when stored in a warm place. That such a difference is due to the condition of the storage, may be seen from the results of our experiments reported in the previous paper. It seems likely that for the cap plasmolysis of Höfler and Döring which corresponds with our types *b* and *c* (especially with the latter), is responsible the condition that the material has had the tendency to perform the tonoplast plasmolysis.

In his Fig. 3 Höfler (1934) has shown the simultaneous occurrence and the partial distribution of the cap plasmolysis (in the peripheral region) and the normal convex plasmolysis (in the inner region) in one and the same epidermal tissue of *Allium*. This difference in distribution of the plasmolysis forms may be attributed to the fact that in the periphery of the pieces of tissue the cells are somewhat injured by being cut and inclined to show the cap plasmolysis of the types *b* and *c*, while in the cells of the inner part the normal conditions are kept undisturbed.

When the Hecht's fibres remain to exist for a long time in the convex plasmolysis, the cap plasmolysis occurs very rarely. It is generally observed that in *Allium* the Hecht's fibres appear in the healthy cells plasmolyzed in a KCl solution, a fact which indicates that the occurrence of the cap plasmolysis means a somewhat unhealthy condition of the cell. The Hecht's fibres and the protuberances can, however, appear simultaneously in the same material.

In the cap in completion, the drops *A* are not found, but mitochondria-like granules are visible, which appear also in the tonoplast plasmolysis. These fine bodies may be classified into three groups: 1. Granules which are relatively large in size, bright in contour and dark in center in the dark field illumination, 2. Particularly small granules, 3. A filament composed by linear linking of many granules belong to groups 1 and 2, and sparkling in the dark field illumination. From observation of the active Brownian movement of these fine bodies, it is assumable that the cap plasm is very low in viscosity. Pekarek (1940) tried to estimate the viscosity of the cap plasm from observation of the Brownian movement. According to him, the viscosity is ten times as high as that of water when the cap

plasm shows not a remarkable swelling, seven times when it swells slightly more highly in degree, and four times in the maximum swelling.

As Kaiserlehner (1939) has pointed out, in certain regions of the cap plasm, namely in the lacuna, in the streak-like line and in the peripheral region of the cap, no granules exist. According to the result of the present investigation, at the first stage of cap formation the lacuna appears in an irregular lobate form in the cap plasm where granules are found scattered (Fig. 19). This lobate lacuna vibrates continuously, increases in size and changes its form to be spherical or ellipsoidal, appearing transparent. The dark field illumination is very advantageous for tracing successively these changes in the lacuna formation and for the observation of lacuna vibration.

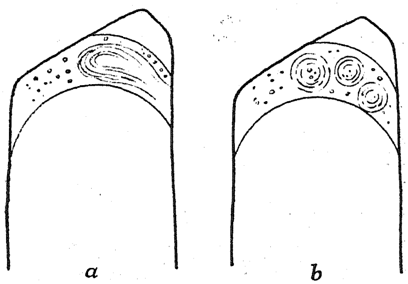


Fig. 19. Material *B*. Somewhat schematised drawings. Lobate lacuna in the cap plasm (*a*). Spherical lacunae in the cap plasm (*b*).

In agreement with Kaiserlehner we are of the opinion that the increase in volume of the lacuna is caused by the osmotic water absorption. The lacuna is frequently liable to confusion with the nucleus. They can, however, be distinguished from each other by the facts that the nucleus does not vibrate and that, according to Kaiserlehner, it is later surrounded by a clear thick membrane. The lacuna seems to have no connection with the drop *A*, but becomes connected with the drop *B*, after its appearance.

The streak-like line is also a certain mass of hyaline consistency which vibrates and shows the changes in size, continually taking place, but we dare not to say positively that it consists of the same material as the lacuna. The outer surface of the cap plasm is sometimes rendered expanded by osmotic water absorption, a phenomenon which appears to be a kind of deplasmolysis.

In extreme cases the periphery of the cap reaches the cell membrane, and the interspace between the cell membrane and the tonoplast is filled with the expanded cytoplasm. This state of the cap plasm is apparently similar to the case belonging to Schema 8, and also to the case of the vacuole contraction. A similar appearance is observed also when the outer periphery of the cap plasm is broken and its contents fill up the interspace.

If the cells, showing cap plasmolysis in a 0,5 mol KCl solution, are transferred into another solution of 0,5 mol KCl containing 5% saponin, the cap plasm dissolves by and by in the peripheral part

becoming obscure and obscure, and at last the whole cap disappears, leaving only the granules on the surface of the tonoplast. Probably, this phenomenon indicates that the fundamental substance of the cap plasm consists mainly of lipoid.

If the main substance of the protuberances is supposed to be lipoid, which probably arises from the cytoplasm by the demixing (Entmischung)[1], it may be assumed that the drop *A* participates in the construction of the cap plasm.

According to Höfler (1939) and Kaiserlehner (1939), when the material, in which the cap plasmolysis occurs, is transferred into another solution of higher concentration, the volume of the cap decreases. These authors have also observed that the cap plasmolysis does not appear in a solution of Ca-salt or the same solution with K-salt in addition, and that the cap formed in a K-salt solution shrinks in these solutions containing Ca-salt. They are of the opinion that such a shrinkage of the cap plasm is caused by colloidal dehydration which is due to the active penetration of Ca-salt into the cap. Kaiserlehner has observed that when the material showing the cap plasmolysis in a KNO_3 solution is treated with a hypertonic $CaCl_2$ solution, a plasmolysis-like phenomenon occurs at the outer periphery of the cap, the phenomenon which he explains by assuming that the plasm at that part is precipitated by the $CaCl_2$ that penetrated.

According to our view, the results of these authors seem to be interpreted without great difficulty, if the whole cap plasm is regarded as an osmotic system and if it is assumed that its periphery is permeable to K-salt, but not to Ca-salt. Under such a condition as assumed, the water movement will take place osmotically, not as a colloidal phenomenon. This interpretation is based also on our own observation that the shrinkage of the cap can take place not only in a Ca-salt solution, but also in a saccharose solution, in which latter case such a dehydrating action as in a colloidal system can hardly be assumed to take place. It may, however, be possible that a membrane-like layer is produced as a result of the reaction between the peripheral substance of the cap and the Ca-salt, as pointed out by Kaiserlehner.

Part II

A. Observations at successive stages of the plasmolysis

In the previous investigation and in the experiments belonging to Part I of the present paper, the etiology of the various types of plasmolysis in the inner epidermis cells of *Allium cepa* was investigated and it was found that they are different according to difference in the characteristics of the matreial.

1) Gicklhorn (1932).

In the investigation belonging to the present chapter the course of the plasmolysis was successively observed, in different kinds of material in the sense of plasmolysis, some different kinds of plasmolyticum being used. The results of the observations are given in Tables 1–6, in which the plasmolysis forms are named in the order of the frequency of their occurrence.

Experiment 1

Material: *A*. Room temperature: 21°C–23°C. (Tables 1, 2 and 3).

Table 1. Saccharose solution (0,8 mol)

Immediately	Concave, (no Hecht's fibre, cytoplasmic movement).
After 30 minutes	Concave, angular.
,, 1 hour	Concave, convex, (cytoplasmic movement).
,, 2 hours	Ditto.
,, 6 hours	Convex, (cytoplasmic movement, accumulation of cytoplasm at the narrow cell end).
,, 24 hours	Convex, (Schema 2), (no cap, cytoplasmic movement).

Table 2. KCl solution (0,5 mol)

Immediately	Concave, (Hecht's fibres).
After 30 minutes	Concave, angular.
,, 1 hour	Convex, concave.
,, 2 hours	Convex, concave, (cytoplasmic movement, systrophe) (Figs. 20, 21).
,, 5 hours	Convex, (cytoplasmic movement in a few case).
,, 20 hours	Cap plasmolysis, (accumlation of cytoplasm at the narrow cell ends without cap formation, dead).

Figs. 20, 21. Material *A*. Several plasmolyzed cells, 2 hours after the beginning of the plasmolysis.

Table 3. $CaCl_2$ solution (0,5 mol)

Immediately	Remarkably concave, (Hecht's fibres thicker than in KCl).
After 30 minutes	Concave.
,, 1 hour	Concave, convex.
,, 2 hours	Concave, convex, (cytoplasmic movement).
,, 4 hours	Convex, (cytoplasmic movement).
,, 24 hours	Convex, (cytoplasmic movement, all alive).

Experiment 2

Material: *B*. Room temperature: 23°C–25°C. (Table 4). Saccharose solution (0,8 mol).

Results: Nearly the same as in the case of material *A*, plasmolyzed in the saccharose solution.

Table 4. KCl solution (0,5 mol)

Immediately	Concave.
After 30 minutes	Concave, convex, accumulation of cytoplasm at the narrow cell end, cap plasmolysis (*a*) in beginning.
,, 1 hour	Ditto.
,, 2 hours	Ditto, cap plasmolysis appears.
,, 3 hours	Convex, (protuberance (3), spherical drop *A*).
,, 20 hours	Dead, cap plasmolysis, convex containing the drop *A*.
,, 24 hours	Dead, tonoplast convex, (Schema 7), dead.

Experiment 3

Material: *C*. Room temperature: 22°C–23°C. (Tables 5, 6 and 7).

Table 5. Saccharose solution (1 or 0,8 mol)

Immediately	No plasmolysis separation, concave, (no Hecht's fibres).
After 10 minutes	No plasmolysis separation, concave, convex, tonoplast plasmolysis, (Schema 4).
,, 2 hours	Convex, (cytoplasmic movement), tonoplast plasmolysis.
,, 3 hours	Convex, (accumulation of cytoplasm at the narrow cell ends).
,, 4 hours	Convex, (drop *A* at the narrow cell ends).
,, 24 hours	Convex, (cytoplasmic movement), no cap.

Table 6. KCl solution (0,5 mol)

Immediately	Tonoplast-concave, (Schema 4), concave, concave (Schema 6).
After 30 minutes	Tonoplast-concave, convex[1], cap plasmolysis (Schemata 4→5).
,, 1 hour	Ditto.
,, 2 hours	Cap plasmolysis, tonoplast-convex, (Schemata 4, 5, 7, 8).
,, 4 hours	Cap plasmolysis, tonoplast-convex increases.
,, 6 hours	Ditto, dead cells increase.
,, 24 hours	Dead, tonoplast-convex.

Table 7. $CaCl_2$ solution (0,5 mol)

Immediately	Concave, (easy transformation to the convex, Hecht's fibres inconspicuous, rest of cytoplasm in interspace .
After 1 hour	Convex, (cytoplasmic movement).
,, 2 hours	Convex, ditto.
,, 4 hours	Convex, ditto.
,, 24 hours	Dead, convex, (cytoplasmic movement).

1) This convex plasmolysis can hardly be regarded as quite normal one.

Weis (1925), Cholodny and Sandkewitsch (1933) have reported that in the epidermal cells of *Allium* a rapid rounding up of the cytoplasm occurs when the cells are plasmolyzed with a KCl solution, while the concave form lasts for a long time in a hypertonic $CaCl_2$ solution. In this experiment they carried out, the latter two authors have in some cases met with an opposite result in regard to the plasmolysis form, but this seems to be due to the arbitrary adoption of the observation time, as pointed out in the previous paper.

According to the results of the above experiments, it may be said that generally a K-salt tends to cause a convex form more rapidly than a Ca-salt. It must be noticed also that when a cell is plasmolyzed in a K-salt solution, the resulting tonoplast plasmolysis might often be taken for a convex plasmolysis, if the microscopical observation is made at a not sufficiently high magnification. The results of our observations may be summarized as follows:

When material *A* (a quite fresh material) is plasmolyzed in a saccharose or a $CaCl_2$ solution, it shows first the concave form of plasmolysis, and after a while, this form is transformed to the convex form which continues for 24 hours, the cytoplasmic movement being still kept active. The cap is not formed, and there are found almost no dead cells even after 24 hours. When the same material is plasmolyzed in a KCl solution, the normal concave form first appears and it lasts for a pretty long time, but not so long as in the case when saccharose or $CaCl_2$ solutions are used, and after 20 hours many cap plasmolyses become observable. Judging from Höfler's result, it seems that in our case the cap plasmolysis begins to appear probably after 6 hours. After 24 hours many dead cells are found in the cells plasmolyzed in a KCl solution.

When material *B* is plasmolyzed in a KCl solution, the cap plasmolysis appears more early than in material *A;* already after 2 hours even it appears. It is a noticiable fact that in material *C* the plasmolysis hardly occurs in a saccharose solution. A similar result is also obtained when the normal material is dried, as seen in the experiments to be described below. When material *C* is plasmolyzed in a KCl solution, the tendency of showing such an abnormal plasmolysis as tonoplast plamolysis or cap plasmolysis is perceived. In the cells plasmolyzed in a $CaCl_2$ solution the normal convex plasmolysis is found with the cytoplasmic movement which lasts for a long time. In a saccharose solution the plasmolytic condition is almost similar to the case of $CaCl_2$ and weak tendency towards the abnormal plasmolysis is shown.

B. Influence of tissue immersion in water on the plasmolysis form

It has been investigated by some authors how the preliminary immersion of the cell materials in water influences on the plasmolysis form[1]. Höfler (1918, 1934) has pointed out that the previous immersion in water accelerates the rounding up of the cytoplasm. Cholodny (1924) has observed that the untreated inner epidermis of *Allium* shows the concave plasmolysis in a solution of a salt of alkali metal (including KCl) as well as in a saccharose solution, while in the material, which has previously been immersed in water for 24–48 hours, the convex plasmolysis occurs in a saccharose solution and the concave in a KCl solution. Kamiya's (1939) results of observation of the cases with and without a previous treatment are as follows: The winter material of *Allium* shows the convex form of plasmolysis and a few Hecht's fibres 20–30 minutes after the beginning of plasmolysis in a 1 mol saccharose solution, if no previous immersion is made. When the material is previously immersed in distilled water for 17 hours, a characteristic plasmolysis with jagged cytoplasmic surface and coarse Hecht's fibres appears after 20–30 minutes. In a 1 mol KNO_3 and in a 0,7 mol $Ca(NO_3)_2$ solution convex plasmolysis appears, if the material is untreated, while the concave form is found in the case of immersed material. When tap water is used as immersion medium, the plasmolysis time is somewhat longer than when distilled water is used as medium, and the negative part of plasmolysis shows a tendency of being extended. In these results, it is recognizable that the influence of immersion is not so remarkable as might be expected[2].

In the present experiments, *Allium* materials of *A*, *B* and *C* classes were used, and the influence of previous immersion of the material in water on the plasmolysis forms was investigated. In case of material of class *C* it was examined with special attention whether such an immersion has any effect on the recovery of the healthy condition.

Rhoeo discolor has frequently been employed as a classical material for the experiment of plasmolysis in laboratory. Thus

1) For the literature we may refer to Kamiya's paper (1939, p. 154). The influence of the immersion on the exosomosis of solutes from a cell is not concerned here.

2) According to Weis (1925, p. 154), in the epidermis of *Allium cepa* which has been previously kept floating on tne surface of distilled water, numerous Hecht's fibres appear and the Brownian movement of granules and the streaming of cytoplasm last for a long time, when the cells are plasmolyzed.

it seems interesting to investigate this problem of immersion also with *Rhoeo*, especially because of the fact that in the older investigations the previous immersion has sometimes been carried out and sometimes not.

In the present experiments tap water and redistilled water were used as immersion media (22°C). The material (epidermis) was immersed with much care not to float on the water surface.

1. *Allium cepa*

The experiments were made in all the seasons of the year with the epidermis of *Allium cepa*. It was previously ascertained by plasmolyzing with a 0,5 mol KCl solution, whether the material, which was either obtained directly from the field (class *A*) or stored (classes *B* and *C*), was in the healthy condition or not.

Experiment 4

Material: *A*. Room temperature: 22°C. Plasmolyticum: saccharose (1 mol) or KCl (0,5 mol). Time of observation: 15 minutes after the immersion in the plasmolyticum. Concave in a saccharose solution, concave in a KCl solution. (Tables 8 and 9).

Table 8. Saccharose

Immersion	Tap water	Redistilled water
1 hour	Concave, convex	Convex, concave
3 hours	Concave, convex	Convex, concave
6 hours	Concave, convex	Convex, concave
24 hours	Convex	Convex

Table 9. KCl

Immersion	Tap water	Redistilled water
1 hour	Concave	Concave, convex (both jagged)
3 hours	Concave	Ditto
6 hours	Concave	Ditto
24 hours	Concave, convex, (both jagged)	Ditto

Experiment 5

Material: *B*. Room temperature: 22°C. Plasmolyticum: saccharose (1 mol) or KCl (0,5 mol). Time of observation: 15 minutes after the immersion in the plasmolyticum. Some cells could not be plasmolyzed easily in a saccharose solution. The plasmolysis showed transitional forms: concave→convex form. Concave plasmolysis in a KCl solution. (Tables 10 and 11).

Table 10. Saccharose

Immersion	Tap water	Redistilled water
1 hour	Concave, convex	Convex, (often coarsely jagged)
2 hours	Concave, convex	Ditto
4 hours	Concave, convex	Concave, convex
24 hours	Convex	Convex

Table 11. KCl

Immersion	Tap water	Redistilled water
1 hour	Concave	Convex, (polar accumulation of cytoplasm), concave
2 hours	Jagged, concave	Ditto
4 hours	Concave, convex (both jagged)	Convex, concave (both jagged), tonoplast-plasmolysis
24 hours	Ditto	Concave, convex (both jagged) Fig. 22

From the results of experiments given above it is seen that water immersion exerts slight influence on the plasmolysis form in materials *A* and *B*, and no remarkable difference is recognizable between *A* and *B*.

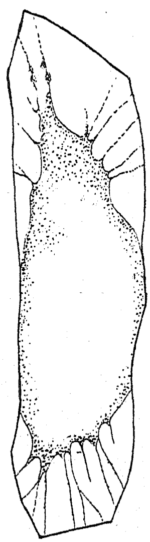


Fig. 22. Material *B*, immersed in redistilled water for 24 hours. Jagged surface of the plasmolyzed cytoplasm. Camera lucida drawing.

The difficulty, with which in the material *B* the plasmolytic separation in a saccharose solution is prevented from taking place, can however, be removed by the water immersion. The immersion in redistilled water acts to promote the convex plasmolysis, and also the same previous treatment with tap water causes this tendency of promoting the plasmolysis in a saccharose solution. Generally the concave form lasts longer in a KCl solution than in a saccharose solution, if the immersion is made in tap water or in redistilled water. The cells plasmolyzed in a saccharose or a KCl solution after the immersion in redistilled water and those plasmolyzed in a KCl solution after the immersion in tap water show their cytoplasm contracted and with a jagged surface, while such is not the case with the cells plasmolyzed in a saccharose solution after the immersion in tap water.

The jagged surface of the contracted cytoplasm is a characteristic effect of the water immersion, and this has been recently investigated in detail by Kamiya (1939). The results we obtained are consistent with those of Cholodny in respect to the point that the immersion produces a greater tendency of presenting the concave plasmolysis in a KCl solution than in a saccharose solution.

If in our case the material of *A* or *B* class of *Allium cepa* was used, the effect appeared merely to such an extent as mentioned above. That in the case of Kamiya the effect of the immersion was generally

of the same degree, as we have seen, seems to be due to the fact that all the materials used by Kamiya were so fresh that they could be grouped into classes as our *A* or *B* material. The same appears to be the case also with the experiments of Cholodny and Sandkewitsch (1933).

When material of *C* class, in which commonly the tonoplast plasmolysis easily occurs, is immersed in water, it may be expected that this tendency of causing the tonoplast plasmolysis with an abnormal easiness can be avoided by this previous treatment. This expectation is shown in the following experiments as a real fact.

Experiment 6

Material: *C*. Some cells were not easily plasmolyzed in a saccharose solution after 15 minutes. The plasmolyzed cells showed transitional stages: concave→convex. Tonoplast plasmolysis or similar abnormal convex plasmolysis occurred in a KCl solution. Room temperature: 22°C. (Tables 12 and 13).

Table 12. Saccharose

Immersion	Tap water	Redistilled water
30 seconds	Concave, not plasmolyzed	Concave, not plasmolyzed
10 minutes	Ditto	Concave, scarcely plasmolyzed
30 minutes	Ditto	Concave
1 hour	Ditto	Concave, convex

Table 13. KCl

Immersion	Tap water	Redistilled water
30 seconds	Concave, (slow plasmolysis)	Concave, (slow plasmolysis)
10 minutes	Concave	Concave
30 minutes	Concave	Concave, convex
1 hour	Concave	Concave, convex

The process of plasmolysis in a saccharose solution is generally slow in *C* material. While this may probably be due to the increased adhesion of the cytoplasm to the cell menbrane, it may also be assumed as an additional reason that under the condition of this increased adhesion the cell membrane becomes somewhat impermeable to saccharose. This resistance to the plasmolytic separation is not to be overcome by water immersion, but it is somewhat reduced, when a KCl solution is used as plasmolyticum.

The most noteworthy fact found in the above experiments is that the tonoplast plasmolysis or a similar tendency observable in a KCl solution is almost completely avoided from its occurrence, if the immersion is made for a short time (30 seconds) in redistilled or tap water. It may, therefore, be said that a remarkable effect of the immersion can be seen, only when material of *C* class is used.

This will be more clearly recognizable in the following chapter where the influence of drying material on the plasmolysis is treated.

In the previous paper it was confirmed that when an onion bulb is kept in a moist place or a scale is immersed in water, before use for the experiment of plasmolysis with a KCl solution, concave plasmolysis can continue for a rather long time, and no tendency of occurring of abnormal plasmolysis, such as tonoplast plasmolysis, is shown. This fact agrees with the results of experiments on the effect of water immersion described above, and the material used at that time seems to belong to our *C* class. If material of *A* or *B* class is used, such a tendency as giving rise to tonoplast plasmolysis is not recognizable at all, and the influence of the immersion in water or storing in wet we is not to be emphasized. The storing in wet itself is a necessary condition in getting the material of *B* class.

The experiments mentioned above are concerned mainly with the effect of water immersion on plasmolysis duration. In the following experiments its influence on the plasmolysis form was observed and the results are shown by the frequencies of occurrence of different types of plasmolysis.

Experiment 7

Material: *B* Concave plasmolysis in a saccharose solution, which occurred after 15 minutes, the plasmolytic separation being difficult. Concave plasmolysis in a KCl solution. Room temperature: 22°C. Immersion: 30 minutes in tap water (22°C). Plasmolyticum: saccharose solution (1 mol) and KCl solution (0,5 mol). Time of observation: 30 minutes after immersion of the material in the plasmolyticum. (Tables 14 and 15).

Table 14. Saccharose

	Concave		Convex		Number of observations
	Number	%-age	Number	%-age	
Not immersed	41	59	28	41	69
Immersed	64	57	48	43	112

Table 15. KCl

	Concave		Convex		Number of observations
	Number	%-age	Number	%-age	
Not immersed	115	97	4	3	119
Immersed	126	95	7	5	133

From these results, it may be seen that any effect of water

immersion on the plasmolysis form is hardly perceivable, if such a fresh material as *B* class is used.

Experiment 8

Material: *C*. Concave plasmolysis including some tonoplast plasmolysis in a saccharose solution after 15 minutes, with plasmolytic separation taking place difficultly. Concave plasmolysis in KCl solution, being accompanied by frequent occurrence of tonoplast plasmolysis. Room temperature: 22°C. Immersion: 30 minutes in tap water (22°C). Plasmolyticum: saccharose solution (1 mol) and KCl solution (0,5 mol). Time of observations: 20 minutes after immersion of the material in the plasmolyticum. (Tables 16 and 17).

Table 16. Saccharose

	No plasmolysis and concave plasmolysis		Convex		Tonoplast plasmolysis		Number of observations
	Number	%-age	Number	%-age	Number	%-age	
Not immersed	96	72	17	13	21	15	137
Immersed	26	19	111	81	0	0	137
Not immersed	91	83	6	6	12	11	109
Immersed	66	50	66	50	0	0	132

Table 17. KCl

	Concave		Convex		Tonoplast plasmolysis		Number of observations
	Number	%-age	Number	%-age	Number	%-age	
Not immersed	20	20	6	7	72	73	98
Immersed	102	95	5	5	0	0	107

In the case of the immersion any tendency toward the abnormal plasmolysis is not observed. In all the cases concave plasmolysis decreases in frequency in a saccharose solution and increases in a KCl solution, when the material was previously immersed in water. In the former solution the transformation from concave to convex is accelerated and in the latter solution the frequency of occurrence of convex plasmolysis decreases as a consequence of the tendency toward the abnormal plasmolysis diminished by the water immersion.

2. *Rhoeo discolor*

Experiment 9

Material: Sections of 3 mm² in size from midrib epidermis of the leaves of a healthy potted plant. Room temperature: 22°C. Immersion: 30 minutes or 18 hours in tap water (22°C). Plasmolyticum: saccharose solution (0,4 mol). Time of observation: 20 minutes after immersion of the material in the plasmolyticum. (Table 18).

Table 18

	Concave and angular		Convex		Number of observations
	Number	%-age	Number	%-age	
Not immersed	42	30	96	70	138
Immersed (30 min.)	65	45	80	55	145
Not immersed	83	73	30	27	113
Immersed (18 hours)	93	85	17	15	110

From Table 18 it may be seen that in the case of *Rhoeo discolor* the influence of immersion in water on the plasmolysis in a saccharose solution is different from that observed in the case of *Allium cepa*. Convex plasmolysis does not increase in frequency by immersion of the material in water, but rather shows a tendency of being decreased.

This remarkable difference from the result obtained with *Allium cepa*, is, at present, difficult to explain. Probably, it may be due to the difference in the nature of the cell membrane between these materials. A certain difference is also found to exist between these two plants, *Allium* and *Rhoeo*, in the experiment of drying effect on the plasmolysis, as will be described below in the following section.

C. Influence of drying and the subsequent immersion on the plasmolysis form

From the facts we ascertained in the preceding experiments it seems not unreasonable to suppose that the unhealthy condition of the *C* material is due, at least in respect of the plasmolysis form, to the loss of water during storage.

It is then to be investigated, how great is the influence of previous drying of tissue on the plasmolysis, and also how remarkable is the effect of the subsequent water immersion of the dried material. For this purpose of investigation the leaves of *Allium cepa*, *Helodea densa*, *Rhoeo discolor* were used as material.

1. *Allium cepa*

Experiment 10

Material: *B*. Room temperature: 22°C. Drying: by exposure of inner epidermis stripped off from the scaly leaf to the room atmosphere on a filter paper for 20 minutes. Immersion after drying: 10 minutes in tap water (22°C). Plasmolyticum: saccharose solution (1 mol) and KCl solution (0,5 mol). Time of observation: 10 minutes after immersion of the material in the plassmolyticum. (Table 19).

Table 19

	Saccharose	KCl
Dried	Plasmolytic separation somewhat difficult. The cytoplasm vacuolated by the violent separation from the cell membrane. Convex or tonoplast-convex plasmolysis.	Plasmo'ytic separation somewhat difficult, but easier than in the saccharose solution. Tonoplast plasmolysis, concave and convex plasmolysis.
Immersed after drying	Many dead cells. The survivors are plasmolyzed with difficulty.	Concave plasmolysis somewhat quickly. Dead cells in more or less number.

Experiment 11

Material: *A*. Room temperature: 23°C. Drying: By exposure of inner epidermis stripped off from the scaly leaf to the room atmosphere on a filter paper for 20 minutes. Immersion after drying: 10 minutes in tap-water (23°C). Plasmolyticum: saccharose solution (1 mol) and KCl solution (0,5 mol). Time of observation: 10 minutes after immersion of the material in the plasmolyticum. (Table 20).

Table 20

	Saccharose	KCl
Dried	Plasmolytic separation somewhat difficult. Plasmolyzed cells showing convex form	Plasmolytic separation somewhat difficult, some cells showing tonoplast plasmolysis
Immersion after drying	Plasmolytic separation easily, concave	Plasmolytic separation quickly, concave

According to these results, when the material is dried, the plasmolytic separation becomes remarkably difficult[1] in a saccharose solution, but not so notably in a KCl solution. This difficulty may partially be due to the increase of the adhesion between the cytoplasm and the cell membrane. But it seems to be also explicable in another way, namely by assuming that the cell membrane becomes more or less impermeable to saccharose by drying, because this impermeability of the cell membrane, has been observed actually to exist by other authors, for instance, in normally growing *Funaria*.

As already explained, the extreme increase of the adhesion is sometimes harmful to the cytoplasm during the plasmolysis, and apt to cause the tonoplast plasmolysis, especially when a KCl solution is used as the plasmolyticum, to which the cytoplasm is more or less permeable.

If the material is as fresh and as healthy in condition as *A* class material, the injury by the drying becomes lesser and a remarkable recovery of the normal state of the cytoplasm takes

1) Schmidt (1939) has found the similar fact in *Lamium maculatum.*

place after the water immersion. If, however, *B* class material is used, the drying effects so a considerable injury that the water immersion is no longer sufficient enough to cause recovery. This injury in the case of *B* or *C* class material, due to drying, sometimes causes the death of cells at the moment of water immersion. It is greater in the case of saccharose solution than in the case of KCl solution. This remarkable fact seems to support Iljin's opinion (1927, 1930, 1933), in which it is maintained that drought injury of plant cell is mainly due to the violent mechanism which occurs between the cytoplasm and cell membrane during the drying or the successive water supply.

The differences, which are seen in *Allium cepa* among materials of different conditions in connection with the plasmolytic separation in a saccharose solution and with the plasmolysis form in a KCl solution, depend after all upon the wet condition during the storage of the bulbs. Also the fact that the water immersion of sections favors the recovery of the normal condition of plasmolysis can be interpreted as the result of a moderate wetting of the boundary region between the cytoplasm and cell membrane. It can also be attributed to the same reason, that the preservation of the scale leaf of *Allium cepa* in cool water is very effective of diminishing the abnormal tendency of the cytoplasm in plasmolysis as described in the previous paper.

2. *Helodea densa*

A more remarkable effect of drying upon plasmolysis may be expected in the aquatic plants. The lower cell layer of the leaf of *Helodea densa* was used for the investigation of the influences of drying and the subsequent water immersion.

The materials were obtained from the pool in a green house. Though it has been reported by some authors[1], that in the growing leaf of this plant the plasmolysis form varies according to the growing zones, such local variations in one leaf may be neglected here, because fully developed leaves were used in the present investigation. In each experiment some number of leaves were taken from the one and same node. No sooner had the water been wiped off from the leaves than a part of them was put in the plasmolyticum, while the other part was dried on a filter paper for a certain number of minutes, before it was subjected to the same immersion.

Experiment 12

Water temperature: 22°C. Drying: for 10 minutes on a filter paper. The leaves slightly shrunk. Plasmolyticum: saccharose solution (0,5 mol) and KCl solution (0,25 mol). Time of observations: 5 minutes after the immersion in the plasmolyticum. (Tables 21 and 22).

1) Strugger (1933, 1935 p. 24-25), Borriss (1938).

Table 21. Saccharose

	No plasmolysis		Concave		Convex		Number of observations
	Number	%-age	Number	%-age	Number	%-age	
Not dried	0 0	0 0	7 15	8 13	77 102	92 87	84 117
Dried	3 6	3 5	61 95	73 81	20 17	24 14	84 118

Table 22. KCl

	No plasmolysis		Concave		Convex		Number of observations
	Number	%-age	Number	%-age	Number	%-age	
Not dried	0 0 0	0 0 0	8 18 24	9 17 21	79 88 90	91 83 79	87 106 114
Dried	0 2 3	0 3 3	65 74 88	75 91 80	22 5 19	25 6 17	87 81 110

From Tables 21 and 22, it is seen that, in *Helodea densa* also, drying makes the occurrence of plasmolysis difficult and prolongs the stage of the concave form, while tonoplast plasmolysis does not appear so quickly in a KCl solution as in the case of *Allium cepa*.

3. *Rhoeo discolor*

As remarked in preceding experiments, in *Rhoeo didcolor* the influence of water immersion on plasmolysis is entirely different from that of the case of *Allium cepa*, the results being rather reverse. The following experiments were made to examine how drying and the subsequent water immersion effect on plasmolysis in this material.

Experiment 13

Material: sections of 3 mm^2 in size from the midrib epidermis of the leaves of a healthy potted plant. Room temperature: 22°C. Drying: by exposing the sections on a filter paper to the room atmosphere until they begin to shrink. Water immersion after the drying: 30 minutes in tap water (22°C). Plasmolyticum: saccharose solution (0,4 mol) and KCl solution (0,5 mol). Time of observation: 20 minutes after the immersion in the plasmolyticum. (Tables 23 and 24).

Table 23. Saccharose

	Concave		Convex		Number of observations
	Number	%-age	Number	%-age	
Not dried	66	71	27	29	93
Dried	37	33	75	67	120
Immersed after drying	71	69	32	31	103

Table 23. (*Continued*)

	Concave		Convex		Number of observations
	Number	%-age	Number	%-age	
Not dried	81	76	25	24	106
Dried	0	0	100	100	100
Immersed after drying	43	32	90	68	133
Not dried	100	100	0	0	100
Dried	25	22	90	78	115

Table 24. KCl

	Concave		Convex		Number of observations
	Number	%-age	Number	%-age	
Not dried	74	69	33	31	107
Dried	0	0	100	100	100
Immersed after drying	45	39	71	61	116
Not dried	59	51	56	49	115
Dried	0	0	100	100	100

As seen from Tables 23 and 24, the influence of the drying is entirely opposite in the case of *Rhoeo discolor* to these found in the case of *Helodea densa* and *Allium cepa*. In *Rhoeo,* the drying of the material increases the frequency of convex plasmolysis, while water immersion after the drying results in a tendency of recovering the occurrence of concave form. It is not easily explainable how the results can be opposite in these cases. A further investigation is necessary to make clear this problem.

Part III

Differences of the plasmolysis form among related species or varieties

From the experimental results on plasmolysis form in *Allium cepa* and some other materials, it is perceivable that the difference in plasmolysis form is due partly to the treatment of the material in the course of the experiment, or to the conditions under which the material plants grow or are stored. It is also noticed that the characteristic of the plant itself has something to do with the plasmolysis form and plasmolysis time. It seems not, therefore, impossible to consider that such differences in physiological characteristics may exist among the species or varieties.

Dehlinghausen (1936) and Kikuchi (1940) have found that there are differences in plasmolysis form among related varieties, which show different degrees of resistance against fungus diseases.

A difference of the plasmolysis form is perceivable between *Helodea densa* and *H. crispa*, while no difference of physiological nature between these plants have not clearely been pointed out[1]. The former plant shows the tendency of presenting the convex form and the latter the concave form in a 0,5 mol $CaCl_2$ solution.

The following experiments were carried out with the view of examining whether any difference of characteristics of presenting definite plasmolysis forms exist among related varieties of *Oryza sativa,* and of *Linum usitatissmum* and among species of *Triticum,* taking the results of the preceding experiments on plasmolysis form into special consideration.

1. *Oryza sativa* (rice plant)

Since the water supply exerts, as we have seen in the above experiments, a great influence on the plasmolysis form, paddy and upland rice plants, which have different ecological characters in relation to water, were used for this purpose of the experiment. The culture varieties used are as follows:

Paddy rice plant. Bōzu Nos. 5 and 6, Kuroge, Oyobe, Date-chikanari, Sakigake.

Upland rice plant. Hokkaiwase, Kairyō No. 13, Ooaoge, Shiheigai.

Both the paddy and upland rice plants were planted in porcelain pots without water outlet or in unglazed pots with water outlet. The former were supplied with as much water as in the flooded field, and the latter were watered every day, keeping the same water condition in the soil as in the upland. They were cultivated in the glass house from June to August, and when they were used for the experiments, the pots were removed to the laboratory, and about one hour after the removal the materials were taken. The sub-epidermal cells of the ligule or auricle, at nearly the same level, were used.

As in the shoot cells of the rice plant plasmolysis hardly occurs in a saccharose solution, a KCl 0,5 mol solution was used as plasmolyticum.

In the first place a comparative study was made between the paddy rice plant Bōzu No. 5 and the upland rice plant Hokkaiwase. As will be seen in detail in the following experiments, the influence of watering during growth on the plasmolysis form is very remarkable. It was, therefore, unreasonable to study the difference between the characteristics possessed by these two groups, if water were supplied to the upland rice plant to as small a degree as in the upland

1) Borriss (1938).

culture. Accordingly, as much water was supplied equally to the upland as well as paddy rice plant as is usual in the flooded field.

Experiment 14

Materials: Bōzu No. 5 (grass length 57 cm), Hokkaiwase (grass length 55 cm). Subepidermal cells of ligule. Condition of cultivation: with sufficient water supply in the porcelain pot (the watering condition in the flooded field). Soil temperature: 22°C after removal to the laboratory. Room temperature: 22°C. Plasmolyticum: KCl solution (0,5 mol). Time of observation: 20 minutes after immersion in the plasmolyticum. (Table 25).

Table 25

	Concave		Convex		Number of observations
	Number	%-age	Number	%-age	
Bōzu No. 5 (paddy rice)	46	98	1	2	47
	86	100	0	0	86
	88	95	5	5	93
Hokkaiwase (upland rice)	4	4	96	96	100
	8	9	84	91	92
	8	7	101	93	109

From this result a difference in the plasmolysis form is perceivable between these two varieties; namely the tendency in Bōzu No. 5 (paddy rice) to be of the concave form and the tendency in Hokkaiwase (upland rice) to be of the convex.

In the following experiments, the soils in the pots were equally dried for two days. The plasmolysis form was then observed, and the same observation was repeated after watering. By this method it was possible to study the influence of the different conditions in water supply on one and the same plant body on one hand, and to study the difference between the characteristics of the two varieties under same watering conditions on the other hand.

Experiment 15

Materials: Bōzu No. 5, grass length 39 cm. Hokkaiwase 32 cm. Subepidermal cells of the ligule. Condition of cultivation: In an unglazed pot. Soil temperature: 23°C in the glass house. After the removal to the laboratory, 22°C. Room temperature: 22°C. Watering: tap water (22°C) was supplied to the dried soil, and after about 30 minutes guttation began. Then the second observation was made. Plasmolyticum: KCl solution (0,5 mol). Time of observations: 30 minutes after immersion in the plasmolyticum. (Tables 26 and 27).

Table 26. Bōzu No. 5

	No plasmolysis		Concave		Convex		Number of observations
	Number	%-age	Number	%-age	Number	%-age	
No watering	100	100	0	0	0	0	100
Watering	4	4	110	96	0	0	114
No watering	100	100	0	0	0	0	100
Watering	11	7	137	93	0	0	148

Table 27. Hokkaiwae

	No plasmolysis		Concave		Convex		Number of observations
	Number	%-age	Number	%-age	Number	%-age	
No watering	0	0	57	93	4	7	61
Watering	0	0	8	9	85	91	93
No watering	100	100	0	0	0	0	100
Watering	0	0	22	7	58	73	83

When the paddy rice plant Bōzu No. 5, which has originally the tendency to present the concave plasmolysis is cultured in dry soil, the plasmolytic separation becomes very difficult. This is due probably not to the impermeability of the cell membrane to the plasmolyticum, but to the increased adhesion between the cytoplasm and the cell membrane, because in this case the plasmolyticum KCl is used, which is regarded as able to penetrate the cell membrane of this material. The effect of watering appeared after 30 minutes, and in the case of Bōzu No. 5 the plasmolytic separation became easier and the concave form increased in frequency, but the convex form was not yet visible.

The similar effect of lack of moisture was found to be true also in Hokkaiwase (upland rice), which shows originally a tendency of presenting the convex form, though the cases where plasmolysis did not occur, were comparatively few in number and rather the concave form increased in stead of the convex. Watering thus decreased the elevated adhesion and permitted the convex form to be presented more easily than in Bōzu No. 5.

It is perceivable that there are tendencies common to these two varieties, a tendency toward the increase of the adhesion in the dry culture and a tendency to decrease it by the watering, though it can not be neglected that the nature proper to the cytoplasm fundamentaly acts to determine the plasmolysis form. This fact indicates how highly important the conditions for growth are, specially the condition of water supply, when tests are made on the difference in the plasmolysis form among related species or varieties. The same statement is applicable also in the case of flax, which will be described later.

In the above experiments the difference of the plasmolysis form between the paddy and upland rice plant was investigated by using only two representative varieties. To see whether similar differences also exist between other paddy and upland rice varieties, the following experiments were carried out.

Experiment 16

Material: (Paddy rice plant) Bōzu No. 5, 34 cm. Bōzu No. 6, 36 cm. Kuroge, 33 cm. Oyobe, 40 cm. Datechikanari, 34 cm. Sakigake, 36 cm. (Upland rice plant) Hokkaiwase, 30 cm. Kairyō No. 13, 27 cm. Ooaoge, 31 cm. Shiheigai, 21 cm. Subepidermal cells of auricle of the second leaf counted from the base. (Ligule was not used, because its development was very variable between the varieties). Conditions of the cultivation: in porcelain pot with sufficient water supply, so as to secure the same condition as in the flooded field. Soil temperature: after the removal of pot to the laboratory 22°C. Room temperature: 22°C. Plasmolyticum: KCl solution (0,5 mol). Time of observations: 30 minutes after immersion of the material in the plasmolyticum. (Tables 28 and 29).

Table 28. Paddy rice plant

	Concave		Convex		Number of observations
	Number	%-age	Number	%-age	
Bōzu No. 5	105 45 74	80 94 77	27 3 22	20 6 23	132 48 96
Bōzu No. 6	100 100 100	88 100 93	13 0 2	12 0 2	113 100 102
Kuroge	48 74 59	55 58 91	40 54 6	45 42 9	88 128 65
Oyobe	44 77 80	67 96 89	22 3 10	33 4 11	66 80 90
Datechikanari	25 70 80	83 90 89	5 8 10	17 10 11	30 78 90
Sakigake	82 54 59	66 93 84	43 3 11	34 7 16	125 57 70

Table 29. Upland rice plant

	Concave		Convex		Number of observations
	Number	%-age	Number	%-age	
Hokkaiwase	32 36 37	35 27 29	59 98 89	65 73 71	91 134 126
Kairyō No. 13	18 46 52	21 29 33	69 111 107	79 71 67	87 157 159
Ooaoge	33 32 6	29 33 6	59 64 100	71 67 94	92 96 106
Shiheigai	36 31 5	31 26 7	76 89 72	69 74 93	112 120 77

It is seen from Tables 28 and 29, that the variation in number of the plasmolysis forms in each variety is pretty great, and it is difficult to obtain a definite number for each variety. Nevertheless, it is recognizable that there is tendency of presenting some definite plasmolysis form, namely that the paddy rice plant has a tendency to show the concave form and the upland rice plant to show the convex form.

Sato (1925) has observed that the parenchym cells of the leaf sheath of the rice plant show convex plasmolysis generally at their growing stage, and rather the concave form at the ripening stage, though in his paper the terms convex and concave have not been used. Since the ligule cells of Hokkaiwase remarkably show the convex form at the growing stage, the cells of the highest ligule on the haulm of this variety were used for the purpose of the examination to see whether the plasmolysis inclines to show the concave form rather than the convex, when the plant grows to be in the ear.

Experiment 17

Material: Hokkaiwase. Subepidermal cells of the ligule, growing just under the spike. Conditions of the cultivation: In porcelain pot with sufficient water supply so as to secure a condition of the flooded field. Soil temperature: after the removal of the pot in the laboratory 23°C. Room temperature: 22°C. Plasmolyticum: KCl solution (0,5 mol). Time of observation: 20 or 25 minutes after the immersion in the plasmolyticum. Control: comparatively young plant not yet in the ear. (Table 30).

Table 30

	No plasmolysis		Concave		Convex		Number of observations
	Number	%-age	Number	%-age	Number	%-age	
After 20 min.	72	80	0	0	18	20	90
After 25 min.	50	77	0	0	15	23	65
Control (after 20 min.)	0	0	88	95	5	5	93

From the table, we see that under the conditions of Exp. 17, the cases showing no plasmolysis increase according to the age of the plants. It may perhaps be due to the increase of the adhesion, that the concave form scarcely appears in the ear stage. This result agrees with those of Sato in the cells of the leaf sheath of this plant. According to him the concave plasmolysis increases in association with ageing of the plant.

In the leaf of *Helodea densa*, it is known that the old cells or the grown up cells are apt to show more frequently the convex form than the growing cells[1]. If reference is made only to the age of

1) Strugger (1933, 1935), Borriss (1938).

cell, this fact would seem to disagree with the present result in the rice plant. It must be, however, borne in mind that in the case of *Helodea* the meristematic stage in the leaf was distinguished from the grown up stage, while in the rice plant the difference of age was mentioned only between the grown up cells. It is, therefore, unreasonable to treat equally both cases.

2. *Linum usitatissimum* (flax)

Dehlinghausen (1936) has found that *Epilobium hirsutum*, which is a species susceptible to *Erysiphe*, has a tendency to show concave plasmolysis, but the hybrid (*Epilobium luteum*×*E. hirsutum* ×*E. hirsutum*× . . . .) which has acquired the immunity, shows rather a tendency toward the convex form. Kikuchi (1940) has made a comparative investigation of plasmolysis form of a culture variety of *Linum usitatissimum* resistant to the wilt disease and of several those which are susceptible to this disease. The resistant material especially used by him was that selected by Dr. Minami from the culture variety "Riga" during its succession cropping. This resistant variety was called "Minami" by Kikuchi in honor of the breeder. The original Riga variety is susceptible to the wilt disease when it is cultivated on sick soil, while the variety Minami shows a remarkable resistance on the same soil. Kikuchi, plasmolysing the epidermal and subepidermal cells of the hypocotyl and the root hairs of flax seedlings with saccharose solution (0,3–0,5 mol), has obtained the following results: Riga and the other susceptible varieties have the tendency to present the convex form, while the variety Minami shows one to present the concave plasmolysis. When the soil temperature rises above 30°C, the result is reverse, that is to say, the susceptible varieties become to show the tendency toward the concave form and the variety Minami toward the convex form, in correspondence with the resistant character acquisition of the susceptible varieties and the loss of this character of the variety Minami.

The writer carried out a series of experiments on this line of question, using some varieties of *Linum usitatissimum*, the seeds of which were kindly granted by the courtesy of Prof. Kikuchi and others of the Hokkaido Agricultural Experimental Station to Prof. Sakamura. The varieties employed in the present investigation were as follows: Minami, Riga, Pernau, France, Holland, Indo, Nansho and a certain Unnamed Resistant Variety (U.R.V.).

The flax plants were cultivated in porcelain pots or in unglazed pots in the glass house. The maximum soil temperature in the glass house was 26°C. The subepidermis of the hypocotyl was used as material. The striping off the thin tissue from the hypocotyl was

made by holding the cotyledon between the fingers and drawing it downwards. Saccharose solution of 0,5 mol or 0,3 mol were used as plasmolyticum. No remarkable difference was found between these two concentrations. The tissue was directly immersed in the plasmolyticum.

In the first place, the examination was made as to whether or not there exists any difference in the form of plasmolysis between the resistant and susceptible varieties.

Experiment 18

Material: 5 days material[1], ca. 5 cm[2], with plumule not yet elongated. Subepidermis of hypocotyl from the plant in the pot kept in the laboratory for 3 hours after the removal from the glass house. Condition of cultivation: water supply sufficient. Maximum soil temperature: 24°C. Soil temperature at the time of the experiment: 21°C. Room temperature: 21°C. Plasmolyticum: saccharose solution (0,5 mol). Time of observation: 10 minutes after the immersion of the material in the plasmolyticum. (Table 31).

Table 31

Variety	Concave		Convex		Number of observations
	Number	%-age	Number	%-age	
Minami	16 11	14 12	99 82	86 88	115 93
Riga	73 89	81 86	17 14	19 14	90 103
Pernau	60 78	90 76	5 24	10 24	67 102
France	70 77	78 84	15 15	22 16	85 92
Holland	65 77	76 81	21 18	24 19	86 95
Indo	48 63	60 66	32 33	40 34	80 96
Nansho	40 31 50	45 38 42	48 46 68	55 62 58	88 78 118
U. R. V.	41 32 19	39 39 23	64 51 63	61 61 77	105 83 82

From the result of Experiment 18, it is readily perceived that the resistant variety Minami has a remarkable tendency toward the convex plasmolysis, while the original susceptible variety Riga and

1) By this expression a seedling, which was sowed 5 days before the experiment, is meant.
2) The length of the seedling.

the other susceptible varieties show a tendency toward the concave form.

The resistant variety U.R.V. is an outcome from the strain of the variety Minami. Perhaps this variety seems to be of a hybrid origin, a hybrid between the variety Minami and a certain unknown susceptible variety. Nansho is a somewhat resistant variety, which has been bred by means of a crossing between U.R.V. and the variety Belgium. In respect of plasmolysis form these two varieties have an intermediate character between the variety Minami and the susceptible varieties.

These results of the experiments we obtained are not in agreement with those of Kikuchi, inspite of the fact that same susceptible varieties as used by Kikuchi were used as material in the present investigation[1]. As mentioned above, the variety Minami has, according to Kikuchi, the tendency of presenting the concave plasmolysis, while the susceptible varieties have shown to be in the tendency toward the convex form. In our case, on the contrary, the variety Minami shows the tendency toward the convex plasmolysis, while the susceptible varieties that of presenting the concave form.

In the case of Kikuchi, the relation between the resistant and susceptible varieties in the plasmolysis form was reversed, when the soil temperature rose above 30°C. In the writer's case the soil temperature was generally 20°–24°C, even the highest temperature not exceeding 26°C in the glass house. Consequently, the discrepancy between these two results of investigation can not be attributed to the soil temperature. An adequate explanation is at present not easy to be made. However, the following point may be important for the consideration of the question. As will be seen from the result of experiments immediately to follow, the plasmolysis form in *Linum* has a close relation to the soil moisture, and its influence appears within a short time. It may not be impossible that the various processes taken during the preparation of the stripped tissue for experiment, such as water immersion, drying etc. will act as factors which are closely connected with the determination of the plasmolysis form. Further experiments are thus necessary to be carried out, keeping these points in mind. It may also not be ignored in connection with the question at issue that the concentration of the plasmolyticum (sassharose) was sometimes different between the case of Kikuchi and ours. Kikuchi generally used 0,3 mol saccharose solution, while we employed 0,5 mol solution. An additional experiment

1) The morphological characters of seedlings of the varieties Minami and Riga perfectly coincide with those described by Kikuchi (1940).

was made to see whether this difference in concentration of the plasmolyticum had anything to do with the discrepancy found between the two cases.

Experiment 19

Material: 7 days material. ca. 5 cm. Minami and Riga. Subepidermis of the hypocotyl. Condition of cultivation: with sufficient water supply. The maximum soil temperature: 21°–24°C during the experiment. Room temperature: 22°C. Plasmolyticum: saccharose solution (0,3 mol). Time of observation: 20 minutes after the immersion of the material in the plasmolyticum. (Table 32).

Table 32

Variety	No plasmolysis		Concave		Convex		Number of observations
	Number	%-age	Number	%-age	Number	%-age	
Minami	8	11	2	2	66	87	76
Riga	29	30	47	48	22	22	98

No difference is recognizable between the results obtained with a 0,3 mol (Table 32) and a 0,5 mol solution (Table 31). It is, therefore, not adequate to attribute the discrepancy between the results of Kikuchi and ours to the difference in concentration of the plasmolytica. If the present result is compared with that obtained by Dehlinghausen with *Epilobium*, it will be equally seen in both these cases that the resistant variety shows a tendency toward the convex plasmolysis, while the susceptible one does another tendency toward the concave plasmolysis, though the pathogenic fungi and the plants used were different in these cases from each other. We obtained the same result in the case of *Triticum*, as will be seen later.

Generally a low soil moisture is unfavourable to the flax in its growing stage and the tendency of being apt to suffer from the wilt disease is shown when the soil moisture is low[1]. This fact seems to indicate that the susceptible variety, which shows concave plasmolysis, is rendered to grow in the soil of high moisture, while the resistant variety, which shows convex plasmolysis, is to grow in nature in the soil of low humidity. The former will be called of the concave or moist type, and the latter of the convex or dry type.

In the case of the rice plant, it was shown above that the water content in the soil greatly influence upon the plasmolysis form. In a series of preliminary experiments with *Linum*, it was often found that the plasmolysis form is liable to change according to the soil moisture, in the stage before the plant shows any sign of wilting.

1) Kikuchi (1940).

In Experiment 20 the influences of the soil drying and subsequent watering on plasmolysis form in the flax were investigated. As material the varieties Minami and Riga were used.

Experiment 20

Material: Minami and Riga, 18 days material. Both ca. 12 cm, showing no sign of wilting. Subepidermis of the hypocotyl. The tissue material taken immediately after the removal of the pot to the laboratory. Condition of cultivation: The soil somewhat dry. Soil temperature: 21°C. Room temperature: 22°C. Watering: sufficient supply of tap water (21°C). Soil temperature: after the watering 20°C. Observation, 30 minutes or one hour after watering. Plasmolyticum: saccharose solution (0,5 mol). Time of observation: 20 minutes after the immersion of the material in the plasmolyticum. (Tables 33 and 34).

Table 33. Minami

	No plasmolysis		Concave		Convex[1]		Number of observations
	Number	%-age	Number	%-age	Number	%-age	
Before watering	20 18	42 38	25 28	52 58	3 2	6 4	48 48
30 minutes after watering	9 9	19 17	17 16	35 30	22 28	46 53	48 53
One hour after watering	2 3	3 7	5 4	9 8	49 41	88 85	56 48

Table 34. Riga

	No plasmolysis		Concave		Convex		Number of observations
	Number	%-age	Number	%-age	Number	%-age	
Before watering	17 12	23 26	55 32	75 68	1 3	2 6	73 47
30 minutes after watering	8 10	18 21	35 36	80 75	1 2	2 4	44 48
One hour after watering	8 3	13 6	47 38	78 78	5 8	9 16	60 49

Experiment 21

Material: Minami and Riga, 7 days material: Both 5,5 cm. showing no sign of wilting. Subepidermis of the hypocotyl. The tissue material taken immediately after removal of the pot to the laboratory. Condition of cultivation: the soil more dry than in the preceding experiment. Soil temperature: 21°C. Room temperature: 22°C. Watering: Sufficient supply with tap water (22°C). Soil temperature after the watering: 22°C. Observation: 30 minutes after watering.

1) Sometimes the tonoplast-convex plasmolysis appeared with a 0,5 mol saccharose solution, when the soil was dry. As it occurred, however, only in a very few cases, it has not been taken into account. If a KCl solution (0,5 mol) had been used, this type of plasmolysis would have occurred more frequently.

Plasmolyticum: Saccharose solution (0,5 mol). Time of observation: 20 minutes after the immersion of the material in the plasmolyticum. (Tables 35 and 36).

Table 35. Minami

	No plasmolysis		Concave		Convex		Number of observations
	Number	%-age	Number	%-age	Number	%-age	
Before watering	3	3	75	80	16	17	94
30 minutes after watering	0	0	12	14	71	86	83

Table 36. Riga

	No plasmolysis		Concave		Convex		Number of observations
	Number	%-age	Number	%-age	Number	%-age	
Before watering	3	4	72	89	6	7	81
30 minutes after watering	2	2	92	87	12	11	106

Experiment 22

Material: Minami and Riga, 15 days material. Both ca. 11 cm. Sub-epidermis of hypocotyl, showing a slight wilting. The tissue material taken immediately after the removal of the pot to the laboratory. Condition of cultivation: The soil very dry. Soil temperature: 21°C. Watering: Sufficient supply of tap water. Soil temperature after the watering: 14°C. Observation, 30 minutes after watering. Plasmolyticum: saccharose solution (0,5 mol). Time of observation: 20 minutes after the immersion in the plasmolyticum. (Tables 37 and 38).

Table 37. Minami

	No plasmolysis		Concave		Convex		Number of observations
	Number	%-age	Number	%-age	Number	%-age	
Before watering	19	31	37	61	5	8	61
30 minutes after watering	2 0	3 0	17 14	29 26	40 40	68 74	59 54

Table 38. Riga

	No plasmolysis		Concave		Convex		Number of observations
	Number	%-age	Number	%-age	Number	%-age	
Before watering	23	31	50	68	2	1	74
30 minutes after watering	9 6	12 8	65 64	84 90	3 1	4 2	77 71

From the results given in Tables 37 and 38, the following points are made out. When the water supply in the soil is not sufficiently

made[1)] the plasmolytic separation becomes difficult and the concave plasmolysis increases in frequency, instead of the convex, inspite of the fact that the flax plant has not shown any sign of wilting. This phenomenon takes place in both cases of varieties Minami and Riga, though in the former it is somewhat less conspicuous than in the latter. When the soil is further more highly dried, a larger number of individuals come to show no longer plasmolysis. However, if the soil is supplied with sufficient water, the tendency of presenting the normal plasmoylsis and the convex plasmolysis becomes recovered, by a 30 minutes watering even. This recovery is conspicuous especially in the case of the variety Minami, but not in the case of Riga, though in this variety too a small tendency of the recovery is perceivable to exist. Such an effect of the dry soil condition may be regarded as due to the increase of the adhesion between the cytoplasm and the cell membrane, and partly to the impermeability of the latter to saccharose. It is admittable that the increased adhesion plays a great rôle in this case, because the same tendency is seen to exist also in the case of plasmolysis with a 0,4 mol KCl solution which is generally regarded as having a free penetrability through the cell membrane, even in a dry condition.

The subepidermal cell of the hypocotyl of *Linum* is one of the most suitable materials for the observation of the change of the plasmolysis form according to the age of the material. In the following Experiment 23 this change was investigated in the flax seedlings in a short duration.

Experiment 23

Material: Minami and Riga. (1) 5 days material, both ca. 3 cm. (2) 15 days material, both ca. 11 cm. (3) 19 days material, both ca. 14 cm. cotyledon yellowish. Subepidermis of the hypocotyl. Material taken 3 hours after the removal of the pot to the laboratory. Condition of the cultivation: Sufficient water supply. Soil temperature at the observation: 22°C. Room temperature: 22°C. Plasmolyticum: saccharose solution (0,5 mol). Time of observation: 10 minutes after the immersion in the plasmolyticum. (Tables 39 and 40).

Table 39. Minami

Age of plants	No plasmolysis		Concave		Convex		Number of observations
	Number	%-age	Number	%-age	Number	%-age	
5 days	0	0	11	11	90	89	101
15 days	0	0	18	25	53	75	71
19 days	17	24	38	52	17	24	72

1) This dry condition of the soil is often the case also in the open field.

Table 40. Riga

Age of plants	No plasmolysis		Concave		Convex		Number of observations
	Number	%-age	Number	%-age	Number	%-age	
5 days	3	4	58	76	15	20	76
15 days	54	58	38	41	1	1	93
19 days	82	82	18	18	0	0	100

From Tables 30 and 40 it is seen that old plants show a tendency toward the concave plasmolysis or not to plasmolyze at all. Especially this was the case with Riga, which has the natural tendency toward the concave plasmolysis. The increase of the concave form in the old material of the flax coincides with the increase which was observed in the case of the rice plant in the preceding experiments.

3. *Triticum* (wheat)

According to Schulz (1913), *Triticum* is systematically classified into three groups, One-grain, Emmer and Vulgare. Besides these groups, Lilienfeld and Kihara (1935) have set up a fourth group Timopheevi. This classification of Schulz agrees well with the results of the serological, pathological and cytological investigations[1].

Table 41

Group	Chromosome number (n)	Reaction to the rust disease
One-grain	7	Immune
Emmer	14	Resistant
Vulgare	21	Susceptible
Timopheevi	14	Resistant

Some experiments were performed to see whether any characteristic difference in plasmolysis form exists between these groups of *Triticum*. As materials the following species were used:

One-grain group: *T. aegilopoides, T. monococcum*

Emmer group: *T. dicoccoides, T. dicoccum, T. durum, T. polonicum, T. Khapli*

Vulgare group: *T. vulgare, T. Spelta, T. sphaerococcum*

Timopheevi group: *T. Timopheevi*

In the first place *T. monococcum, T. durum* and *T. vulgare* were selected as material representing each of these groups. All the plants were of the same age, and the plasmolysis form was observed in the parenchym cells of the inner subepidermis of the second leaf sheath, especially in the part near the ligule.

Experiment 24

Condition of cultivation: pot culture in the glass house. Moderate water supply. Soil temperature: 22°C, one hour after the removal of the material to

1) Reference is made to Kihara (1938).

the laboratory. Room temperature: 22°C. Plasmolyticum: KCl solution (0,5 mol). (Table 42).

Table 42

After immersion in the plasmolyticum	*T. monococcum*	*T. durum*	*T. vulgare*
7 minutes	Convex	Convex, angular, concave	Angular, concave, no plasmolysis
10 minutes	Convex	Convex, angular, concave	Angular, concave, no plasmolysis
15 minutes	Convex	Convex, slightly concave	Angular, concave, convex

T. monococcum presented the most remarkable tendency toward the convex form and *T. durum* followed it in this tendency, while *T. vulgare* showed a tendency rather toward the cancave plasmolysis or did not plasmolyze at all. This difference may be attributed to the degree of the adhesion, and the magnitude of the difference is expressed in the order: *T. monococcum* $<$ *T. durum* $<$ *T. vulgare*.

In the following experiment, many species of wheat being used, a comparative examination of the characteristics of their cytoplasm, indicated by the plasmolysis form, were attempted. The materials were kindly granted from Prof. Kihara in Kyoto, which were cultivated in May and June in his experimental field.

Experiment 25

Plasmolyticum: KCl solution (0,5 mol and 1 mol) and saccharose solution (1 mol). Time of observation: 20 minutes after the immersion of the material in the plasmolyticum. (Table 43).

Table 43

Group	Species	KCl (0,5 mol)	KCl (1 mol)	Saccharose (1 mol)
One-grain	*aegilopoides*	Convex	Convex	Convex
	monococcum	Convex	Convex	Convex
Emmer	*dicoccoides*	Convex	Convex	Convex, concave
	dicoccum	Concave, convex	Concave, convex	Concave, convex
	durum	Convex, concave	Convex, concave	Concave, convex
	polonicum	Convex, concave	Convex, concave	Concave, convex
	Khapli	Convex, concave	Convex, concave	Concave, convex
Vulgare[1]	*vulgare*	Concave, convex	Concave, convex	No plasmolysis, concave, convex
	Spelta	Concave, convex	Concave, convex	No plasmolysis, concave, convex
	spherococcum	Concave, convex	Concave, convex	No plasmolysis, concave, convex
Timopheevi	*Timopheevi*	Many tonoplast plasmolysis		Convex, tonoplast

1) On the whole plasmolysis proceeded slowly and showed its tendency not to occur.

The result of the preceding experiment was thus confirmed by the results of the present experiment in which the order of the adhesion: One-grain group < Emmer group < Vulgare group, were more definitely generalized than in the preceding experiment.

In the point that the more resistant the plant is to fungus diseases, the more the plasmolysis has a tendency toward the convex form, the result in *Triticum* agrees very well with those in *Linum* and *Epilobium*[1], admitting that the pathogenic fungi are naturally different in these cases.

Among the Emmer group, *T. dicoccoides* showed the convex form almost as easily as *T. monococcum,* while *T. dicoccum* showed the most conspicuous tendency toward the concave form. This fact would mean that these species occupy certain special systematical position, hence have characteristic natures in the Emmer group to the rust disease. *T. Timopheevi,* which is regarded as comprised in a special group, showed characteristically tonoplast plasmolysis, but it is almost impossible to consider at present any relation of the plasmolysis form to exist to the systematical or pathological nature of this group.

Part IV

General Discussion

1. *Etiology of the tonoplast plasmolysis and cap plasmolysis*

The result obtained in the previous and present investigations on the etiolgy of the tonoplast plasmolysis and cap plasmolysis in *Allium cepa* may also apply in the case of the other plants. In the previous paper (Takamine, 1940a), general processes of the plasmolysis of these kinds were shown in photographs and schemata. To these eight schemata we may now add another one, the ninth.

Schema 9. In the beginning, a vacuole contraction[2] occurs which is apparently similar to Schema 7. This is followed by a gradual separation of the cytoplasm from the membrane. Thus results a plasmolysis which is equal to tonoplast plasmolysis (Schema 4) or to cap plasmolysis in all appearance. The vacuole contraction appears similar to tonoplast plasmolysis, but its occurrence is not always limited in the case of hypertonic solutions. According to Gicklhorn and Möschl (1930), this occurs as a consequence of active water absorption in swelling of the cytoplasm

1) Dehlinghausen (1936).
2) Küster (1927), Henner (1934).

from the vacuole. The vacuole contraction and tonoplast plasmolysis are, however, phenomena very intimately related, and both may commonly be caused under the conditions that the semipermeability of the ectoplasm is somewhat lost, while that of the tonoplast is kept perfect. Therefore, as the cause of these abnormal types of plasmolysis, factors which reduce the semipermeability of the ectoplasm must be considered, though the nature of the cytoplasm itself has also to do with them. This is seen to be the case, for instance, when K-salts are used as the plasmolytica on the cells of *Helodea canadensis* subjected to vital staining with neutral red[1] or on the epidermal cells of *Allium cepa* dried or exposed to ultra-violet rays[2].

As described by Höfler, the cap plasmolysis can be derived from a convex plasmolysis followed by the swelling of the cytoplasm at the narrow cell ends (cap plasmolysis *a* of the present case). It also seems possible to consider that other types *b* and *c* of cap plasmolysis can be derived from the tonoplast plasmolysis. In fact we can follow the processes successively in *Allium cepa*. Höfler (1939) is, it seems to us, also of the opinion that the cap plasmolysis is related in its occurrence to plasmolysis of other types. As a common point to the cap plasmolysis and to the vacuole contraction he recognizes that at first a swelling of the cytoplasm occurs, then the separation of a phase rich in lipoid from it on the surface of the vacuole, the fluid tonoplast being strengthened thereby. It seems very probable that there exists such a common point between these phenomena, if an intimate relationship is recognized in their occurrence as mentioned above.

Weber (1930, p. 106) has observed that in the cells of *Helodea canadensis* the vacuole contraction occurs when they are vitally stained with neutral red, and there results, through a plasmolytic treatment, a form which appears very similar to cap plasmolysis. It is very probable that a similar process takes place in the extreme case of tonoplast plasmolysis *a* in *Allium cepa*.

From what we have considered above, it may be concluded that the tonoplast plasmolysis results in variation in degree according to the intrability of the cytoplasm to the plasmolyticum in combination with the surface tension of the tonoplast. de Haan (1934) has mentioned that there may exist a certain relation between the cap plasmolysis and the vacuole contraction in *Allium cepa*, but he has not made any observation in detail.

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1) Weber (1930).
2) Takamine (1940b).

2. *Factors which influence the plasmolysis form*

The factors can be classified as follows:

a) Conditions during the process of plasmolysis.
b) Factors, prior to the plasmolysis, acting on material during its treatment, including the storage.
c) Characteristics of the material plant.

a) Conditions during the process of plasmolysis

First of all mention may be made about the plasmolyticum. It is without question that the saccharose solution is the most suitable plasmolyticum in determining the isotonic concentration of the critical plasmolysis, but this is not always the case when the experiment concerns the plasmolysis form. In some materials, for instance in the leaf or ligule of *Gramineae* plants, plasmolysis hardly occurs in the saccharose solution, but even the cytorrhysis. This difficulty of the plasmolytic separation is due to the impermeability of the cell membrane to saccharose, and in the case of such materials a KCl solution must be used.

Similarly, when the characteristic of the cytoplasm is examined by the plasmolysis form method, a KCl solution may be preferred to a saccharose and a $CaCl_2$ solution in some materials, for instance in *Allium cepa,* because the KCl solution has a peculiarity to disclose the nature of the cytoplasm by its strong penetrability. It is a well known fact that the K-, Na- and NH_4-salts act to liquefy the cytoplasm, while the Ca-salt acts rather antagonistically against these salts. Such an antagonistic action of the Ca-salt is also perceivable in relation to the plasmolysis form, that is to say, the easy transformation of the concave plasmolysis to the tonoplast or cap plasmolysis, a transformation which is caused by salts of alkali metals, is effectively prevented by the Ca-salt. A similar action is observable also in Al-salt. These characteristic action of the Ca-salt and salts of alkali metals are visible only when such a healthy material as the *A* class of *Allium cepa* is used. Otherwise it might happen that a contrary result is obtained[1].

Also the concentration of the plasmolyticum has influence upon the plasmolysis form. This influence is simply demonstrated when a saccharose solution is used, because it has no such characteristic effect on the cytoplasm as the ionic actions. In the previous paper it has been shown that in *Allium cepa* the plasmolysis time is shorter

1) The result of Chołodny and Sandkewitch (1933) different from the common belief in the actions of the above mentioned salts on the plasmolysis form has been discussed in the previous paper (Takamine 1940a).

in a 0,6 mol saccharose solution than in a 1 mol solution, a fact which is contrary to the common assumption that a solution of higher concentration would cause plasmolytic separation more quickly. It must be taken into consideration, however, that in plasmolysis there occurs a dehydration of the cytoplasm itself besides the withdrawal of water from the vacuole. It is very probable that in a saccharose solution of extremely high concentration the increase of the adhesion makes the plasmolytic separation difficult as a result of such a dehydration.

The pH value of the plasmolyticum has to do with plasmolysis form and plasmolysis time, especially when it is above 7,0[1]. For this reason it is expected that alkaline cover glass and slide glass may influence the plasmolysis and especially facilitate the occurrence of tonoplast or cap plasmolysis. In the present as well as in the previous investigation it was shown that the production of the protuberance is remarkably promoted by the use of alkaline glass.

In the previous paper the influences of temperature and light on plasmolysis have been shown to be pronounced. Especially the latter one has been shown to be so an important factor in the experiment of the plasmolysis form that it must not be neglected on any occasion. It is, therefore, here recommended that the microscopical observation, which is especially unavoidable in the determination of the plasmolysis time, must be practiced under a strict restriction. In the present investigation the ordinary method of the determination of the plasmolysis time was not applied[2], but the number of the plasmolysis form was quickly determined after certain periods of time and was expressed in %-age.

b) Factors, prior to the plasmolysis, acting on the material during its treatment, including the storage

In the previous paper (1940 a) it was found that the condition during the storage of the onion bulbs has influence upon the plasmolysis form. As the materials obtained from a market have been commonly stored under various conditions, it seems rather natural that the results of experiments, carried out with such materials, may not be quite in agreement.

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1) Errata: In Résumé 3 in the previous paper (Takamine, 1940a, p. 322) for "...... the liquefying action of $K^{\cdot}$ on the cytoplasm increases accompanied by the *increase* of hydrogen ion concentration" is to be "...... increases accompanied by the *decrease* of hydrogen ion concentration."

2) In the previous paper (1940a) the term "plasmolysis time" has been used in explaining the result obtained, but it seems now more suitable to substitute this term by "concave plasmolysis time."

A primary importance is to be attached in these cases, to the humidity, and it must be remembered that the cytoplasm is very sensible to the dryness, at least in regard with the plasmolysis form. This relation of the humidity to the plasmolysis form was confirmed in the experiments with *Allium cepa*. It was also observed that in the rice and the flax plant the water supply through the root exerts much influence upon the plasmolysis form in the shoot cell. These results show that much more the condition of humidity, under which the tissue material is treated before and during the process of plasmolysis, must be taken into account.

There occurs a remarkable difference in the plasmolysis form according as the section may be immersed in the plasmolyticum directly or after a water immersion, even a water immersion for so short a time as 30 seconds being very effective. In the dry condition the adhesion between the cytoplasm and the cell membrane is increased, and in *Allium cepa* abnormal plasmolysis such as tonoplast or cap plasmolysis is caused, when a KCl solution is used as plasmolyticum, because this salt easily penetrates into the cytoplasm. When an attempt is made to reveal the characteristic of the cytoplasm in a certain material by the observation of the plasmolysis form, special attention must be paid to the water relation in the plant body during its vegetation.

The disagreement of the experimental results between Kessler (1935) and Kessler and Ruhland (1938) on one hand and Scarth and Levitt (1937) on the other hand seems to be due to the unequal experimenting procedures. While the former authors directly observed the plasmolysis form in a hypertonic $CaCl_2$ solution, while the latter authors first immersed the material in an isotonic $CaCl_2$ solution and then observed the plasmolysis form after a slight evaporation of water from the plasmolytic solution. The preliminary immersion of the material in an isotonic $CaCl_2$ solution in the latter author's method must correspond with the treatment, the previous water immersion, in the present investigation. It may not be difficult to suppose that such a treatment, which was not employed by the first named authors, caused the plasmolysis to result in different forms.

c) Characteristics of the plant material

The plasmolysis form depends also on the characteristics of species and variety, sometimes of the individual. In the present investigation this relation was studied in the rice plant, flax and wheat.

As the water relation during the vegetation has much influence upon the plasmolysis form, it may be expected that a remarkable

difference is observable in this connection between the paddy and upland rice, which have different ecological character from each other in the water relation. In fact, it is perceivable that under the same flooded condition of cultivation a tendency toward the concave form exists in the former variety, while a tendency toward the convex form in the latter which is able in nature to grow even without irrigation. When the water deficiency occurs in the culture of the paddy rice plant the adhesion between the cytoplasm and the cell membrane becomes increased, and this renders the plasmolytic separation difficult. Microscopical observations indicate that this is an actual fact.

According to Iljin (1927, 1930, 1933) the wilting injury of the plant cell takes place during the time when the cell wilts or the wilting cell is supplied again with water, because a violent mechanical force here acts between the surface of the cytoplasm and the cell membrane. If this opinion is accepted, it will then become possible to consider that an increase of the adhesion strengthens such a violent action as Iljin assumes and will make the injury more severe. Under this consideration it is natural that the plant in which the plasmolysis is very apt to begin with the convex form, for instance the upland rice, is more wilt-resistant than the plant in which the concave form is apt to be kept, like the paddy rice.

We are not attempting to explain the causal relation between the resistance to the wilt disease and the characteristic of the protoplasm of the flax by the comparison of the plasmolysis form. As the flax demands, however, a pretty sufficient water supply for its favourable growth, and as the flax wilt is caused by *Fusarium Lini* in rather low soil moisture, it would not be improbable to assume that the strong tendency of the wilt-resistant variety Minami to show convex plasmolysis indicates, to a certain extent, an intimate relation between the resistance against the wilting and the resistance to diseases.

In connection with the immunity or the resistance against fungus diseases, the same tendency in the plasmolysis form is visible also in the case of wheat, as already reported by Dehlinghausen (1930) in *Epilobium*. It is thus very desirable to make clear the relation between the wilt-resistance and the disease-resistance in these plants, taking the plasmolysis form into consideration.

Résumé

1. According to the condition of the health, the bulbs of *Allium cepa* are classified in three groups: *A*, *B* and *C*. Those belonging to class *A* are most healthy, and show the normal concave and convex plasmolysis. Those of class *C* are most unhealthy and apt to show

the tonoplast plasmolysis. Those of class *B* occupy intermediate situations in these respects.

2. The tonoplast plasmolysis, which appears in *Allium cepa,* is classified according to its etiology. Generally the occurrence of the plasmolysis of this form is fundamentally due to the fact that the intrability of the cytoplasm increases, while the semipermeability of the tonoplast is maintained unaltered. The increased adhesion between the cytoplasm and cell membrane further facilitates its occurrence.

3. There are at least three ways through which cap plasmolysis results. The characteristics of the protuberance are described in detail and its relation to the cap formation is discussed.

4. Successive stages in the plasmolysis in *Allium cepa,* caused with saccharose, KCl and $CaCl_2$ solution, are followed extending through several hours. The plasmolysis proceeds differently according to the kinds of the plasmolyticum as well as to the conditions of the material.

5. A preliminary water immersion of the material influences the plasmolysis form. In *Allium cepa* this influence is not so remarkable in the case of the healthy material as in the case of the unhealthy one. The tonoplast plasmolysis, which is very common to occur in the unhealthy material in a KCl solution is easily avoidable by the immersion in tap water for a length of time, even so short as 30 seconds, which gives rise to the increase of the frequency of concave plasmolysis.

6. In *Rhoeo discolor* the influence of the water immersion, contrary to that observed in *Allium cepa*, increases the occurrence of the concave plasmolysis in a saccharose solution.

7. In *Allium cepa* and *Helodea densa,* the drying of tissue makes the plasmolytic separation difficult, and facilitates the occurrence of the tonoplast plasmolysis. A subsequent water immersion is effective in avoiding this abnormal tendency of the plasmolysis. In *Rhoeo discolor* a contrary result is obtained also in this respect and the drying increases the convex plasmolysis.

8. The cells of the ligule or auricule in *Oryza sativa* are plasmolyzed with a 0,5 mol KCl solution. The paddy rice has the tendency to show concave plasmolysis and the upland rice convex plasmolysis. The soil moisture shows a quick and remarkable influence on the plasmolysis form of these cells. When the soil is dry, the concave plasmolysis increases and the plasmolytic separation is rendered difficult. The same tendency appears also after the plant is in the ear.

9. The subepidermal cells of the hypocotyl of *Linum usitatissimum* (flax) are plasmolyzed with a 0,5 mol saccharose solution.

The variety Minami, which is resistant to the wilt disease caused by *Fusarium Lini*, shows a more remarkable tendency to convex plasmolysis in comparison with several susceptible varieties, including the variety Riga from which the variety Minami has originated.

10. Also in the case of the flax the same relation between the soil moisture and the plasmolysis form is perceivable as in the rice plant. By a sufficient water supply the normal convex plasmolysis is recovered from the tendencies toward difficult plasmolytic separation and toward concave plasmolysis which are caused by the drying of the soil.

11. In the flax seedling the frequency of concave plasmolysis increases according to the age. A remarkable difference is visible even in a disparity of only a few days in age.

12. Among the strains of wheat a difference of the tendency of the plasmolysis form is visible. The One-grain group inclines to show convex plasmolysis, the Vulgare group toward concave plasmolysis and toward difficult plasmolytic separation, while the Emmer group is intermediate between these. In the Timopheevi group the tonoplast plasmolysis is remarkable apt to occur.

13. A relation between the wilt-resistance and the disease-resistance on one hand and the plasmolysis form on the other hand, is discussed in the case of rice, flax and wheat plants.

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Heterochromosome Formation in *Benzoin aestivale* (L) Nees.

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There are over fifty recorded cases of sex chromosomes in the Angiosperms. New instances of sex chromosomes are still needed in order that we might discover their origin and estimate their full significance. The diversity of opinion as to the exact nature of these heterochromosomes is in itself witness to this need. Among several native Angiosperms which had not received thorough cytological investigation, the author came upon the peculiar reduction division in the pollen mother cells of *Benzoin aestivale* (L) Nees., the common spice bush of eastern North America.

Buds were collected during middays of early September on bushes growing on the Asheville Farm School property adjacent to the Swannanoa river. Material collected later in September and in October of previous years proved to be too far developed to include stages of meiosis. Material from Belmont, Massachusetts collected in 1931 was only useful for comparative studies of pollen. The material from Massachusetts and that collected in 1938, 39, and 40 in North Carolina were preserved in Carnoy's 6-3-1 fluid. The better preservation was obtained in 1940 with a fluid recommended by Dr. Edward C. Jeffrey of Harvard University made of equal parts of absolute alcohol and glacial acetic acid. The buds were immersed, the air withdrawn by means of a vacuum pump, and allowed to stand for several hours. Thereafter they received the usual treatment for the hot nitrocellulose method of imbedding, Jeffrey 1928. Sections were stained with Heidenhain's iron-alum haematoxylin and allowed to decolor very slowly so that the maximum differentiation might be obtained. Zeiss equipment was used for the microscopy and the drawings were made without the aid of a camera lucida.

Microsporogensis. — The nuclei in the PMCs are comparatively small. So are the chromosomes, and therefore no extensive study of the prophase was made. During the diplonema stage many of the chromosomes would be linked in chains of varying length. During diakinesis, (figs. 11 and 12), twelve haploid chromosomes are rather easily distinguished, the elements of which lie well separated. A few of the chromosomes appeared to have already undergone disjunction and some pairs were not easily recognized as such

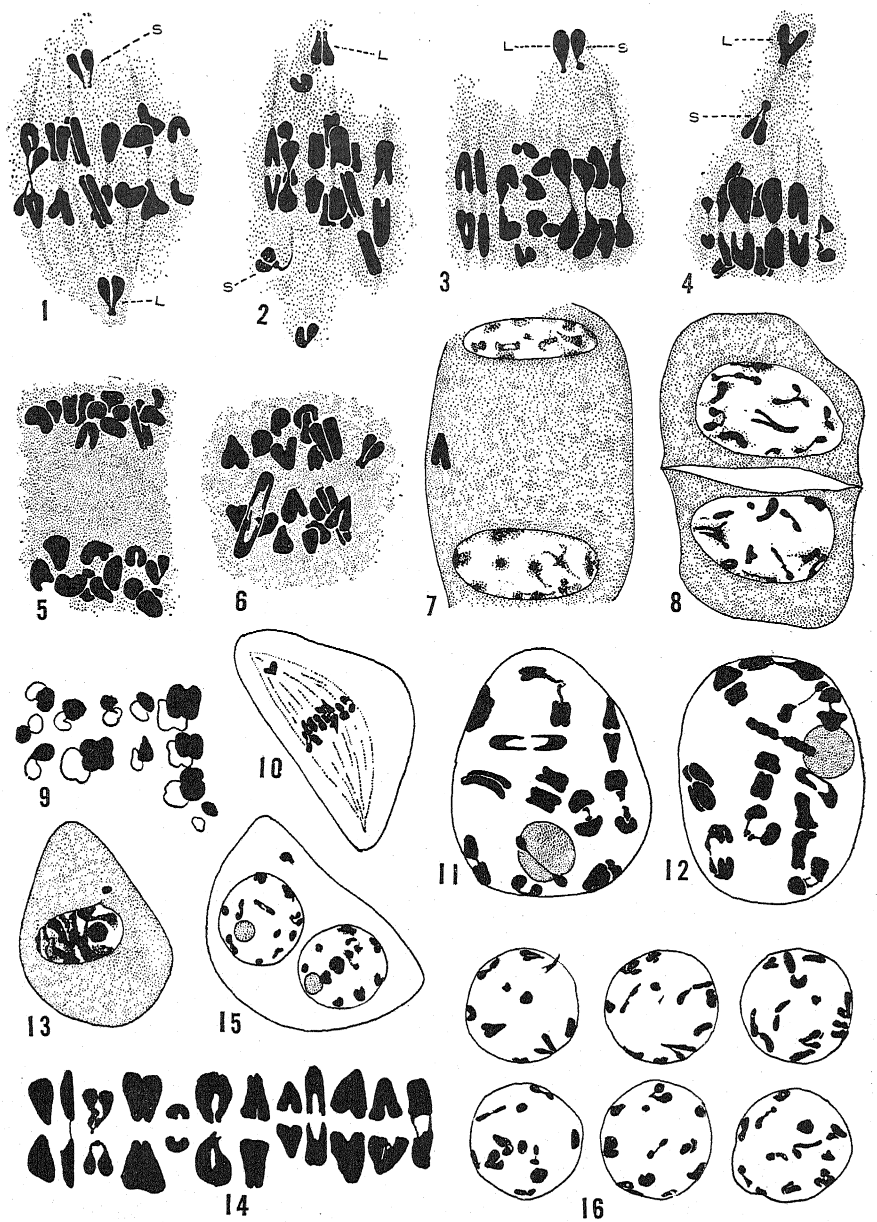
because the two units had become twisted in axial relationship to each other and gave the appearance of two distinct types of chromosome. The pair near the center of figure 12 illustrates this point. There is no distinct metaphase in the heterotypic division. When the nuclear wall dissolves the reduced number of chromosomes lie scattered about the PMC and by the time the majority align themselves near the center, the anaphase is quite in evidence, figure 1 is quite typical. Occasionally a PMC is found in which there is uniformity enough to permit an accurate count from the polar position (fig. 9) —, here again the number is found to be n=12. Figure 14 was drawn from a side view of an early anaphase, though the alignment has been improved upon, and displays the remarkable variation in size and shape of the chromosomes. The sequence from left to right was maintained as found in the cell. There seem to be two extraordinarily large chromosomes, five medium sized, four somewhat smaller, and one exceedingly small pair.

Relatively few PMCs possess a simultaneous distribution of the chromosomes during anaphase of the heterotypic division (fig. 5). Over seventy five percent of the PMCs were irregular at this stage and in some anther sacs all cells seemed to be abnormal. Figure 2 represents the more extreme cases in which two or more pairs had anticipated the migration toward the poles of the spindle. Usually the irregularity was largely confined to one of the medium sized chromosomes. It would divide before the others and the members might go to opposite poles (fig. 1) or to the same pole (fig. 4) or fail to disjoin and lie out in the cytoplasm beyond the spindle (fig. 3). In approximately half of the PMCs the whole or the half of this odd chromosome would remain in the cytoplasm after the daughter nuclei had been formed consequent to the first meiotic division. The author could find no evidence that any but this particular chromosome ever failed to be included in the nuclei, regardless of the number of chromosomes which migrated independently of the main group. Cytokinesis is of the successive type and it was noted that the formation of the new cell wall was, or seemed to be, delayed by the presence of the odd chromosome in the cytoplasm (fig. 7) because in the same anther sac one would find completely divided cells (fig. 8) with no extruded chromatin in the cytoplasm and undivided cells (fig. 7) containing extruded chromatin. However, one might also find stages running from "metaphase" to late anaphase in the same sac during the first meiotic division and this variability may simply have been carried forward to the telophase. Nonetheless, there was a difference between those with and without extruded chromatin.

The second or homeotypic division proceeded without irregularity

except in such instances where the odd chromosome had been included in the nucleus formed from the first division. After disjunction the chromatids would often migrate towards the poles ahead of the other chromosomes, (fig. 10). Although the odd chromosome, or one of its units, might have been extruded in the first division, it was not found to undergo further division in the cytoplasm. It could often be found in the cytoplasm of the microspores (fig. 13) where it seemed to undrgo degeneration. The microspore shown in figure 13 was chosen because it exhibits a condition sometimes observed wherein a small protrusion from the nucleus was extended towards the odd chromosome in the cytoplasm. Figure 16 is a group of six nuclei from microspores showing that some have twelve chromosomes and others only eleven; the deficiency presumably due to the loss of the odd chromosome. The mature pollen from collections made in Massachusetts and North Carolina contain very few imperfect spores. The author was at first bewildered because the walls of the microspore expand rapidly even though there seems to be very little cytoplasm present. The nuclei were slumped against the inner wall and though the spores were not collapsed one got the impression that it was simply a matter of time before the protoplasm would degenerate and the spore walls would implode. That opinion proved to be wrong. The density of the cytoplasm increased during later stages. There seemed to be little basis for any contention that the microspores which were deficient in chromatin aborted while those whose nuclei possessed the normal amount survived.

Because the meiotic abnormality in *B. aestivale* chiefly concerns but one chromosome special attention was given to the size and shape of this body. From time to time there seemed to be a noticeable difference in the appearance of the two halves of this chromosome; either lying side by side (fig. 3) or apart (fig. 4). This difference was not always discernible. A search was made of early heterotypic anaphases in an effort to follow the behavior of the odd chromosome more closely. As shown in fig. 14, third chromosome from left end, the pair is made of two units. One unit, the smaller, possesses deeper constrictions in the two arms of the V which make it a tripartite body. The slightly larger unit is also tripartite but the middle piece is very small and the constrictions more shallow so that the unit often gives the impression of being two-armed with a single and median constriction. During disjunction the odd chromosome sometimes produces a short anaphase bridge, similar to those observed in several species of *Rumex,* Jensen 1936, and at the breaking point, one of the pair seems to get more chromatin out of the bridge than the other. Sometimes the 'tail' still drags in the spindle (fig. 4).



Figs. 1–16. *Benzoin aestivale*; microsporogenesis. 1–6. Heterotypic anaphase showing various aspects of the heteromorphic chromosome pair indicated by S for the smaller unit and L for the larger. 5. Normal anaphase. 7. Larger unit of heterochromosome lying in cytoplasm at end of first meiotic division. 8. Cytokinesis of normal reduction division. 9. Polar view of heterotypic anaphase. 10. Homeotypic metaphase displaying irregular L chromosome. 11, 12. Diakinesis showing heteromorphic chromosomes. 13. Young microspore with extruded chromosome in cytoplasm. 14. Haploid chromosome complement taken from early heterotypic anaphase. 15. Telophase of homeotypic division showing extruded chromosome. 16. Six nuclei from microspores showing varying chromosome complements.

The larger, the less tripartite, of the two elements of the odd chromosome is the portion more often excluded from the daughter nuclei. At least, the chromosome remaining in the cytoplasm resembles the two arm unit and for the most part is larger than the smaller member of the heterochromosome complex as found during the early anaphase, (fig. 7). In addition to a common difference in size and shape, the two members also give evidence of a difference in behavior. However, in keeping with the facts one must be reminded that these differences are not visible in every PMC. Only when conditions were favourable for intimate study of the odd chromosome did these differences seem conclusive.

An effort was made to check the somatic cells to determine the presence of two unlike chromosomes belonging to the same haploid complex. Because the chromosomes are so small no valid statement can be made at this time. It is regrettable that *B. aestivale* should provide this handicap since it offers some interesting possibilities for further study.

Discussion. – If the peculiar meiosis in the PMCs of *Benzoin aestivale* has any significance, its obvious direction would be the influence of hybridization on the origin of sex chromosomes. The existence of laggards as well as early disjunction during the heterotypic division, the extrusion of chromosome material from the spindle and the consequent deficiency in some of the microspores, and the numerous stages of meiosis present in the PMCs of the same anther sac, all these are characteristic of hybrids and now generally accepted, together with other abnormalities, as being diagnostic of hybrid ancestry. Because the meiotic abnomalities are not severe enough to disrupt the process as a whole, there is reason to believe that such hybridization has not been recent. It is interesting to note that Small (1933), who usually can be depended upon to include all known forms, only lists *B. melissaefolium* (Walt.) Nees. in addition to *B. aestivale* as native to eastern North America. Whether or not these two species represent the surviving products of a remote period of hybridization is a question which invites further study. Regardless of the cytological condition of the remainder of the genus, *B. aestivale* gives evidence of previous hybridization which has produced an unusual meiotic behavior in the history of one of its 12 haploid chromosomes.

If the system of Engler-Diels (1936) is followed, the seven species of *Benzoin* which Small recognizes are included in the genus *Lindera*. Here *B. aestivale* becomes *L. Benzoin* (Blume). Most of the sixty species of *Lindera* are found in eastern Asia, Engler-Diels

(1936). Sugiura (1936) found the haploid number of *Lindera glauca* to be 12, which is the same as for *B. aestivale*. In fact, 12 is the only number which the author could discover for any member of the Lauraceae reported in the literature. Täckholm and Søderberg (1917) reported 12 as the reduced number for *Cinnamomum Sieboldi*. The identical number is given for *C. Camphora*, *C. japonicum*, *C. Loureirii*, and *Lindera glauca* by Sugiura (1936).

Studies of microsporagenesis described in an earlier section of this paper have convinced the author that a heterochromosome complex is present during meiosis. As to a permanent disparity which could be verified among the somatic chromosomes, that is another matter. As was admitted, a search among the stages of somatic mitosis was not fruitful because of the very small size of the chromosomes in the material under observation. Of more immediate significance is the effort on the part of the odd chromosome to accentuate the small difference in size and shape of its two component parts, by two types of behavior. The first was the occasional delayed disjunction of the two members with the subsequent formation of an 'anaphase bridge' which, when it did break, often left one member with less chromatin material than the other. The second idiosyncrasy was the rather consistent tendency on the part of approximately 50% of the PMCs to include only the smaller member in one of the daughter nuclei and leave the larger member to degenerate in the cytoplasm of the eventual microspores. The origin and proportion of each of three general types of pollen grain are illustrated in table 1. The smaller and larger member of the heterochromosome complex are symbolized by the letters S and L respectively.

Table 1. Origin of three types of pollen grain in *B. aestivale*.

Behavior of L chromosome	Haploid chromosome composition of PMCs	Composition of daughter nuclei of first div.	Composition of daughter nuclei of second div.	Approx. percent of pollen	Percent functional
L chromo. extruded	11+S–L	11	11	25%	--
			11		
		11+S	11+S	50%	66.6%
			11+S		
L chromo. included	11+S–L	11+S	11+S		
			11+S		
		11+L	11+L	25%	33.3%
			11+L		

The figures quoted are approximations because, as stated in the descriptive material, the degree of abnormality varied from one anther sac to another. It is also quite probable that an occasional pollen grain may contain 13 chromosomes, 11+S+L; although such cases were not observed. This probability is projected because both elements of the odd chromosome were sometime seen at the same pole of the heterotypic anaphase spindle. Since the chromosome number of the species remains 12, the deficient pollen must not succeed in the process of fertilization. Two thirds of the remaining pollen grains contain nuclei bearing the smaller member of the odd chromosome. Whether or not this strength in numbers accomplishes a proportional number of fertilizations is another matter. One should also remember that it is quite possible that the disposition of the egg nuclei may effect a change in this ratio. An attempt was made to count the male and female shrubs in the area from which cytological material had been collected. Several obstacles make a dogmatic statement inadvisable. In the first place, the plants do not come into flower at the same age because it was observed that a number of large bushes bore no flowers. Consequently their sex could not be determined. During autumn and winter the sex of plants which will flower the following spring is quickly determined by the buds. The flower buds on the male bushes usually come in clusters of two and three while those on the females are single and smaller. One can hardly judge by the presence and absence of fruit. While some female shrubs were well provided with fruit, others bore but one or two, even though the buds were distinctly typical of the female. According to some manuals, *B. aestivale* is polygamous-dioecious. Among the shrubs investigated in the Swannanoa valley no evidence of otherwise male bushes bearing fruit was found and considerable search for functional stamens among the female flowers proved equally unsuccessful. Perhaps another year and season might provide the necessary stimulus for the production of mixed flowers. However, it seems safe to conclude that in this particular region *B. aestivale* is functionally dioecious.

One may favour the generally accepted theories relative to heterochromosomes and their role in the determination of sex, so ably summarized by C. E. Allen (1940), or may be inclined to take exception to part or the whole proposition. Nonetheless, heterochromosomes do exist and there is impeccable evidence that a peculiar relationship exists between the members of the heterochromosome complex and the two sexes. Regardless of one's opinion as to their significance, the origin of these bodies needs explanation. Darlington (1937) does not seem to advance any generally satisfactory hypothesis

for the origin of sex chromosomes although he suggests that fragmentation and interchange will account for the transition from one type of heretochromosome to another. Except for increased regularity in behavior, these sex chromosomes, according to Darlington, resemble the behavior of certain chromosomes in some hybrids. It was not clear whether this opinion could be extended backward to cover the origin of heterochromosomes from identical autosomes. Allen (1940) considers the whole question as "often discussed" and "open." Darlington's recognition that there is a similarity between the behavior of certain chromosomes in hybrids and various types of sex chromosomes would seem to be sound and worthy of further consideration, and, in the author's estimation, will prove to be the key to the solution.

Since the sex chromosomes are usually the only heterochromosome complex present in the complement of each sex, one of the intermediate stages between this and the primitive condition wherein no heterochromosomes are present would resemble that found in *B. aestivale*. Here there is a slight heteromorphism in one haploid chromosome which is accentuated by delayed disjunction and maldistribution of one or both of its elements. Although female plants were not investigated, the consistent presence of the larger portion of the odd chromosome in material from a number of plants, despite a 2–1 ratio in favor of pollen bearing the smaller chromosome, indicates that there is reason to believe that the male and female chromosome complements are not identical. A still more primitive stage was found by the author (1939) in the PMCs of *Ilex opaca*. Here again there was a frequent extrusion of one chromosome into the cytoplasm. However, there was no evidence in this case that the chromosome was heteromorphic. Of course, the above suggestion still leaves the general question of heterochromosomes quite open. Interest in establishing a sound explanation must undoubtedly rest upon the desire to investigate a multitude of organisms in various stages of sexual differentiation and genetic background. The report on the cytology of *B. aestivale* is contributed as another case history with a suggestion as to its appropriate place in an evolutionary pattern which may eventually describe with adequacy the origin of sex chromosomes.

Summary

A cytological study of *Benzoin aestivale* revealed the presence of several deviations from the usual meiotic process during microsporogenesis. These irregularities were considered to be diagnostic

of previous hybridization of the species. The principal abnormality involved one of the twelve haploid chromosomes which appeared to be heteromorphic in both size and shape. In approximately half of the PMCs the larger of the heterochromosome elements was extruded from the heterotypic spindle and allowed to degenerate in the cytoplasm. As a result of the unequal distribution of the elements of the heterochromosome approximately two thirds of the functional pollen grains were calculated to carry the smaller element of the heterochromosome complex. The absence of a satisfactory degree of regularity seemed to exclude this case as being another X–Y sex chromosome mechanism. The meiotic behavior of the heterochromosome did suggest that it may represent an intermediate stage in the evolution of what are conventionally called sex chromosomes.

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List of new publications

received by the Journal and the Editor **from July 1940 to June 1941.** Works not belonging to cytology and allied subjects are included only to a limited extent.

Liste der Neuerscheinungen

die der Zeitschrift sowie dem Herausgeber **von Juli 1940 bis Juni 1941** zugegangen sind. Arbeiten, die sich nicht auf die Zytologie und ihre Grenzgebiete beziehen, konnten nur in beschränkter Zahl aufgenommen werden.

Liste des publications nouvelles

reçues par notre Revue et par son Directeur **depuis juillet 1940 jusqu'a juin 1941.** Le travaux, qui ne concernent pas la cytologie et les sujets connexes, peuvent y être inclus, mais en nombre limité.

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